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DYNAMIC THYROID FUNCTION STUDIES

DEVELOPMENT AND APPLICATION OF DYNAMIC TEST
PROCEDURES USING IODINE-131 AND TECHNETIUM-99m
FOR THE EVALUATION OF THYROID FUNCTION

by

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PREFACE

The studies presented in this thesis were carried out from 1969 to 1973 in the laboratories, wards and clinics of the University Department of Medicine, Royal Infirmary, Glasgow. The detailed planning of the work and its execution were my individual responsibility and except where specifically indicated in the text, the work was entirely personally performed.

Selected aspects of the work described in this thesis have been (or are to be) published as original papers in

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Papers based on the work have been read personally to the following learned groups.

London Thyroid Club, 1970.

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SECTION 1

INTRODUCTORY

Chapter 1	Introduction
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Chapter 4	Simulation of thyroidal iodide metabolism

CHAPTER 1

INTRODUCTION

My personal interest in thyroid physiology, initially aroused by Professor E.M. McGirr during my undergraduate tenure of a Carnegie research scholarship, was later fostered by the opportunity of working with Drs. J.A. Thomson and W.R. Greig in the University Department of Medicine, Glasgow Royal Infirmary. The work reported in this thesis was performed while I was in turn a registrar, then a lecturer in medicine and finally an honorary senior registrar.

At that time, in vivo studies with radioactive iodine had provided a firm basis for the scientific evaluation of thyroid function for two decades. Currently, the development and widespread application of radioimmunological techniques has quietly revolutionized the routine investigation of patients with suspected thyroid disease and in particular, in vitro measurements of serum thyroxine, triiodothyronine and thyrotrophin provide indices of thyroid function which are both highly discriminating and more convenient to patient and clinician. Although the adoption of specific hormone assays heralds autumn for the routine evaluation of thyroid status with radioactive iodine, the in vivo approach is necessary when information on the thyroid metabolism of inorganic iodide is required. Such data may be relevant to both diagnosis of thyroid disease and assessment of anti-thyroid treatment effectiveness.

Paralleling advances in radioimmunology, the increasing sophistication of radionuclide imaging devices will now permit an

accurate analysis of rapid changes in thyroid radioactivity with time (kinetic analysis). This thesis has explored the use of in vivo kinetic analysis with a directional counting system and by investigating thyroïdal iodide transport and organification, has attempted to predict areas of diagnostic and therapeutic potential for future development.

The experimental section of the thesis opens by defining the important physical problems in measurement of thyroid radioactivity and continues with an account of the development and assessment of a directional counting system designed specifically to provide accurate quantitative information on rapidly changing radioactivity in thyroid. The random and systematic errors associated with the use of this technique are subsequently studied in detail.

The next section of the thesis has as its theme, the assessment of thyroïdal iodide transport using technetium as pertechnetate. Studies using diffusible autoradiography clearly confirm the similar handling of pertechnetate and iodide by the thyroid transport mechanism and justify the application of a conventional thyroid mathematical model to pertechnetate kinetics in thyroid. Human in vivo experiments which consolidate and expand existing knowledge of thyroid physiology of pertechnetate are described thereby permitting a critical assessment of the value of pertechnetate for clinical investigation. Experiments which explore one potential application of pertechnetate in the treatment of human thyroid disease, namely its use to predict the adequacy of radioactive iodine therapy for thyrotoxicosis are also described.

The thesis then goes on to expand the kinetic theory of radioactive iodide organification in thyroid. From this basis, a sensitive clinical test for detecting inorganic iodide in thyroid is developed, namely the intravenous perchlorate test. Combining in vivo human studies with rat experiments, evidence which argues that thyroidal iodide transport is rate-limiting for thyroid hormone synthesis in human but not in rat thyroid is presented. An extension to the mathematical treatment of iodide organification in thyroid permits presentation of a theoretical analysis for calculating the organification rate of inorganic radioactive iodide in thyroid.

Knowledge of the effect of thyroid disease on iodide organification is obtained in depth using the intravenous perchlorate test. The procedure is shown to be a useful adjunct to measurement of serum anti-thyroid antibodies for the diagnosis of Hashimoto's disease and, in addition, a large proportion of patients with simple goitre are clearly shown to have an unexplained defect of iodide organification in thyroid. Investigation of thyroidal iodide organification following treatment of thyrotoxicosis with radioactive iodine-131 or 125 provides clear evidence that iodine-125 is more effective for selectively inhibiting function at the apex of thyroid follicular cells. This finding confirms current views on the microdosimetry of each radionuclide in thyroid.

Finally, an unusual case report of reversible, inherited hypothyroidism is presented.

The role of kinetic analysis in the investigation and therapeutics

of human thyroid disease has not been fully defined but the present work indicates the scope for such studies and establishes areas for future development.

PLAN OF THESIS

The thesis is presented in one volume. Appendix 1 contains supporting tables; appendix 2 contains an account of standard methods; appendix 3 contains detailed mathematical derivations. References are presented at the end of the thesis.

CHAPTER 2

THE IODIDE TRANSPORT AND ORGANIFICATION MECHANISMS OF THE THYROID GLAND : A REVIEW

Historical Introduction

Goitre was recognised by man before the Christian era (Rolleston, 1936) and its empirical treatment with seaweed or burnt sponge documented from as early as the twelfth century (Matinovic and Ramalingaswami, 1958). Following the report by Davy in 1815 that seaweed was rich in iodine, Coindet (1824), a doctor in Geneva, first suggested a relationship between goitre and iodine and attempted iodide supplementation in his goitrous patients. The resulting violent opposition to iodine therapy from his medical colleagues soon forced him to abandon this experiment. Despite the subsequent demonstration by Chatin (1852) of iodine deficiency in soil and water of endemic goitre areas, it was four decades before Bruns (1894) and Reinhold (1894) reaffirmed the usefulness of iodide supplementation in treatment of goitre. In 1895, Bauman conclusively demonstrated a high concentration of iodine in thyroid and Oswald (1899), a chemist from Zurich, later confirmed a high iodine concentration in thyroglobulin. Isolation of thyroxine by Kendall (1915) and elucidation of its chemical structure by Harrington (1926, 1927), firmly established the role of iodine in thyroid metabolism but further major advance was impeded by inherent limitations in the chemical method.

Following the epoch-making discovery of radioactive iodine by

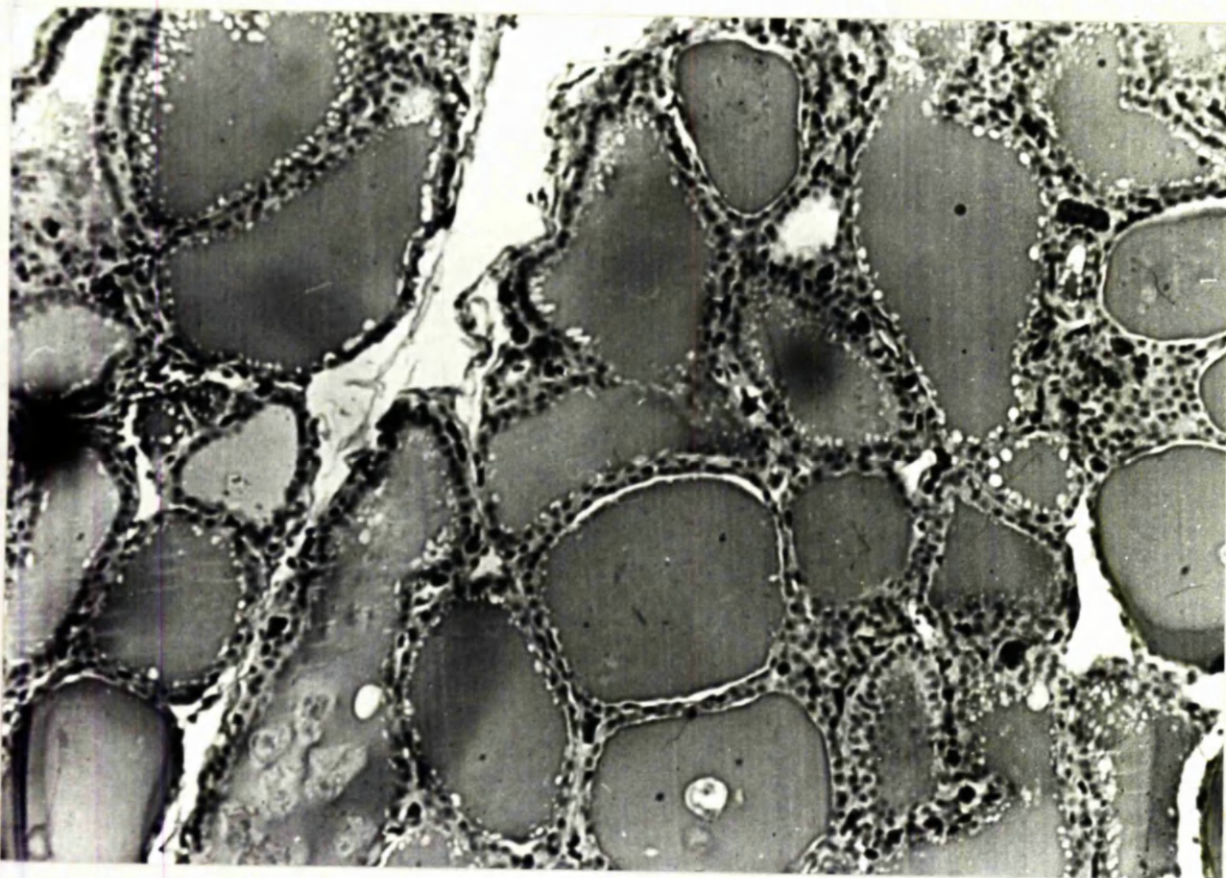


Figure 1: The follicular arrangement of normal human thyroid.

Fermi in 1934, it was soon appreciated (Hamilton, 1938) that radioactive forms of iodine were chemically indistinguishable from the stable form and that observation of the metabolism of this measurable radioactive label would illuminate the intricate pathways of body and thyroidal iodine. Publication of the original studies of thyroid function with ^{128}I (Hertz et al, 1938) and ^{131}I (Hamilton and Soley, 1939) was followed by a prolonged period of research into the structure and function of the thyroid and its hormones. The background knowledge on fundamental aspects of thyroid function which has accrued since then is collated in the remainder of this chapter.

Basic Morphology and Physiology of the Thyroid

The thyroid gland consists of two lobes lying laterally against the trachea and connected by the isthmus anterior to the second and third tracheal rings. Its average weight in the non-goitrous adult is 20 g. The morphological unit is the follicle, made up of a single layer of epithelium surrounding a mass of homogeneous colloid comprised mainly of thyroglobulin (Figure 1). The follicles appear isolated from each other (Isler et al, 1968) and, though the mean follicular diameter is 300 μ (Means et al, 1963), considerable heterogeneity in both follicle and follicular cell size has been clearly demonstrated (Wollman, 1965).

Electron microscopy has revealed the ultrastructure of thyroid follicular cells (Heimann, 1966) and confirmed the presence of an uninterrupted follicular basement membrane and microvilli at the apical cell surface projecting into colloid.

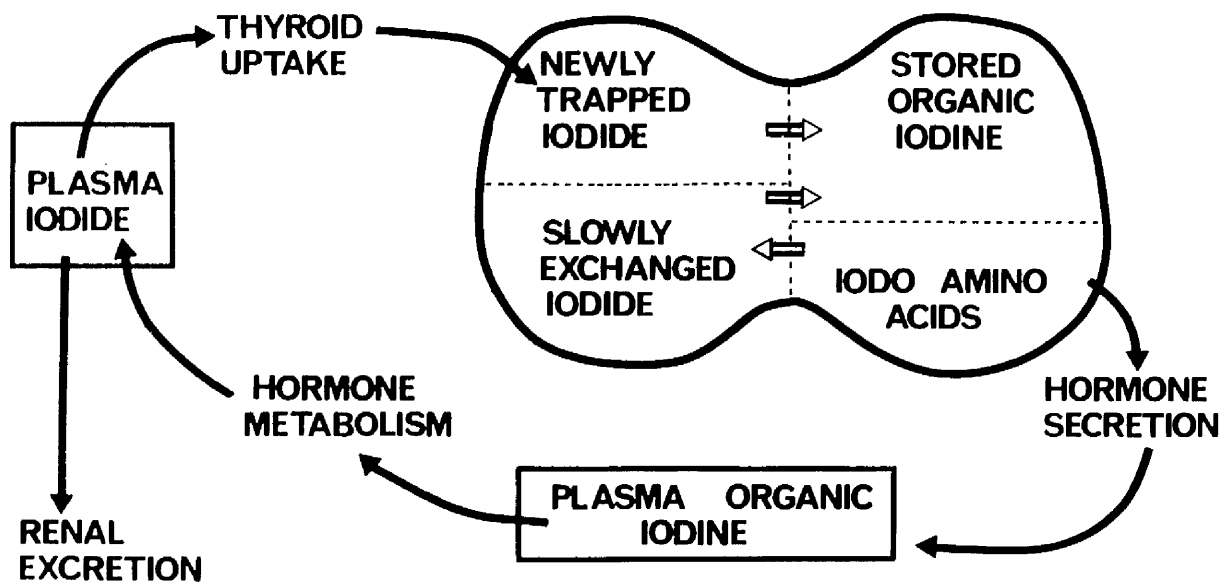


Figure 2: The cycle of iodide metabolism.

Dietary iodide is absorbed from the upper gastrointestinal tract and circulates in plasma as free inorganic iodide (Figure 2) (Myant et al, 1950). Some plasma iodide is excreted by the kidney (McConahey et al, 1951). The remainder is accumulated by thyroid, oxidised, then bound to tyrosine by displacement of hydrogen from the 3 position of tyrosyl residues in peptide linkage in thyroglobulin with formation of mono and di-iodotyrosine (Tong, 1971). Thyroxine and triiodothyronine, formed by coupling of iodotyrosine molecules (Johnson and Tewksbury, 1942), are subsequently split from linkage with thyroglobulin by enzymatic proteolysis (McQuillon et al, 1961) and released into plasma as active hormones. Following hormonal de-iodination in body tissues, released iodide is re-cycled back into the plasma iodide pool (Pitt-Rivers, 1967).

The Iodide Transport Mechanism

Iodide forms only $3 \times 10^{-5}\%$ of igneous rock and there is a similar concentration in normal soil (Moeller, 1952). Human and animal thyroid has shown a remarkable adaptation to this environmental scarcity by developing an efficient iodide concentrating mechanism. Labelled hormone can be demonstrated in functioning thyroid only seconds after exposure to radioactive iodide (De Groot and Davis, 1961). This concentrating mechanism can be conveniently divided into iodide transport and iodide organification by the chemical dissociation achieved using a thiocarbamide drug (for example, carbimazole). This drug prevents organification of accumulated iodide but allows iodide transport to continue (Schachner et al, 1944).

Site of Iodide Transport

Astwood (1944) and Vanderlaan and Vanderlaan (1947) noted that under chemical inhibition of iodide organification, the thyroid concentrated radioactive iodide- ^{131}I from plasma. Pitt-Rivers and Trotter (1953) demonstrated that under similar conditions, thyroidal iodide transport resulted in concentration of inorganic ^{131}I inside the follicular lumen. Doniach and Logothetopoulos (1955b) and Wolff and Maurey (1963) showed that intact thyroid cell membrane was a prerequisite for iodide concentration, and suggested that the active transport mechanism was placed at the apical margin of the cell. Subsequent observation (Tong et al, 1962) of iodide concentration by isolated thyroid cells, which presumably had neither apical nor basal regions, later tended to confirm the general impression that basal membrane was the primary site of iodide transport in vivo.

Further evidence for this has been obtained from the reports of Williams and Vickery (1965) and Andros and Wollman (1967), who both localised ^{131}I in follicular cells of mice at short time intervals after radionuclide administration and clearly demonstrated transfer of cellular ^{131}I to the follicular lumen at later intervals.

The position has been partially clarified by observations of Woodbury and Woodbury (1963). Using a micro-electrode technique, they found the interior of thyroid follicular cells to be 50 millivolts negative to both perifollicular tissue and follicular lumen. This potential difference makes an active transport mechanism for iodide mandatory and implies subsequent iodide diffusion down the electro-

chemical gradient into follicular lumen and colloid. The report by Wolff (1964) that iodide transport is sensitive to external K^+ concentration would certainly corroborate the electrochemical theory.

This electrochemical 'model' provides an explanation for the ability of thyroid to maintain a concentration of inorganic iodide in the follicular lumen, twenty times higher than plasma in normal subjects and several hundred times higher in thyrotoxicosis (Derson and Yalow, 1955). When iodide organification is blocked, the magnitude of thyroidal accumulation of ^{131}I is essentially the resultant of ^{131}I influx and efflux. Evidence indicates that influx is effected by the iodide transport mechanism while efflux depends on iodide back-diffusion against the electrochemical gradient. In addition to their blocking effect on iodide transport (Wynngaarden et al, 1953), perchlorate and thiocyanate magnify this back-diffusion from the follicle (Scranton et al, 1969) possibly by an effect on the electric potential of cell membrane mediated by K^+ loss (Wolff et al, 1968; Young et al, 1970).

There is now abundant evidence that iodide released from intra-thyroidal de-iodination of iodotyrosines forms a biochemically distinct and functionally separate thyroidal iodide pool from iodide transported into the gland from plasma (Nagataki and Ingbar, 1963; Simon, 1963). At the present time, the role of this 'second pool' iodide is largely unknown.

Mechanism of Iodide Transport

Freinkel and Ingbar (1955) and Slingerland (1955) confirmed that

thyroidal iodide transport was an active process and not simple diffusion by demonstrating its dependence on oxidative phosphorylation. Wyngaarden et al (1953) observed saturation kinetics with iodide and competitive inhibition by thiocyanate, perchlorate and other monovalent anions. The iodide transport mechanism also accumulates bromine (Yagi et al, 1953), astatine (Shellabarger et al, 1954) and other members of periodic group 7, namely rhenium, manganese, and technetium, all as the peroxyanion (Baumann et al, 1956). Anbar et al (1959) believe that the existence of thyroid transport for these varied anions is determined mainly by their physical properties (i.e. size, shape and charge) rather than by their chemical nature or periodic relationship. Pertechnetate in particular has a molecular volume and configuration similar to the heavy iodide atom (Wolff, 1964). It seems plausible therefore that a single specific membrane site is involved in transport of all accumulated anions.

Control of Iodide Transport

Halimi et al (1963) have conclusively shown that thyrotrophin is the most important regulator of thyroidal iodide transport. The report (Wilson et al, 1968) that thyrotrophin stimulation of iodide transport could be blocked by inhibitors of protein synthesis such as Actinomycin D, and that V_{max} (the velocity of reaction with fixed enzyme concentration but high substrate concentration) of the transport mechanism was increased without altering K_m (the substrate concentration required to yield half the maximum velocity), suggests that thyrotrophin acts by inducing an enzyme or enzymes which augment the capacity of the

transport mechanism for iodide.

In Vivo Assessment of Thyroidal Iodide Transport

The most precise index of thyroid transport of iodide is the thyroid unidirectional clearance of ^{131}I , a rate-constant proportional to the number of ^{131}I atoms transported into the gland per minute (Rall et al, 1964). The standard thyroid clearance of Keating et al (1949) and Myant et al (1949b), however, measures the net result of ^{131}I influx (transport) and efflux (back-diffusion). It is an accurate approximation of the unidirectional clearance because iodide transport in most subjects appears to be the rate limiting factor in hormone synthesis. There is consequently little inorganic ^{131}I in normal thyroid available for diffusion (Rall, 1956). When inorganic ^{131}I is present in the gland (e.g. pharmacologic block to iodide organification), diffusion reduces the accuracy of net clearance measurements for assessing the transport function. Unidirectional clearance of ^{131}I is mandatory in this situation and its measurement involves use of kinetic analysis (Berson and Yalow, 1955).

Larsson (1955) first recognised that the simply measured 15 - 20 minute ^{131}I uptake of thyroid was little affected by the presence or absence of a pharmacologic block to iodide organification and was therefore an index of thyroidal iodide transport. Using this test he demonstrated excellent discrimination between euthyroid and thyrotoxic subjects. Following confirmation of these findings by Vanderlaan (1957) and Higgins (1959), Thomas (1960) suggested that the 15 minute ^{131}I uptake of thyroid should allow assessment of thyroid

function during antithyroid drug therapy for thyrotoxicosis. Alexander (1969) used this test successfully to measure thyroid suppressibility in thyrotoxic patients on carbimazole therapy.

The concepts of thyroid clearance and early uptake will be enlarged upon in later chapters.

Clinical Defects of Thyroidal Iodide Transport

A defect of thyroidal iodide transport is the rarest inborn error of thyroidal iodine metabolism. First described by Federman (1958), only three additional cases have since been studied in detail (Stanbury and Chapman, 1960; Gilboa, 1963). Criteria for diagnosis include a low thyroidal ^{131}I uptake with a low salivary-plasma and gastric juice-plasma ^{131}I ratios.

The Iodide Organification Mechanism of Thyroid

A recent change of emphasis in thyroid research away from iodine metabolism and towards a more biochemical approach to hormone synthesis has thrown new light on many aspects of thyroidal iodide organification.

Site of Iodide Organification

The precise site of iodide organification remains an enigma though it is now accepted that tyrosyl residues bound on peptide linkage to thyroglobulin are iodinated, rather than free tyrosine which is later incorporated into thyroglobulin (Alexander, 1964; Cartouzou et al, 1964; Seed and Goldberg, 1963; Soodak et al, 1964). Citing evidence from thyroid autoradiographic studies, Leblond and Gross (1948) championed the view that thyroglobulin is formed and iodinated in thyroid follicular cells and subsequently extruded into

the follicular lumen. This view was challenged by Nadler (1954) who demonstrated slow formation of thyroglobulin but rapid appearance of organified ^{131}I in colloid. His suggestion that iodination occurred primarily in colloid in association with microvilli was in accordance with the findings of Stein and Gross (1964). The recent demonstration by Taurog (1970) of a peroxidase enzyme in association with the apical cell membrane coupled with a complete absence of iodinating enzymes in the soluble portion of thyroid constituents including thyroglobulin (De Groot, 1962), argues for the cell membrane as site of iodination. Since isolated thyroid cells have been shown to iodinate external protein (Paston, 1961), the indications are that follicular cells synthesize and secrete thyroglobulin into the follicular cell lumen where iodination occurs while the molecule is in contact with the apical cell membrane (Nadler, 1965).

Mechanism of Iodide Organification

Morton (1943) first demonstrated formation of iodotyrosines in vitro using a preparation of thyroid slices. Subsequent investigators have preferred thyroid-cell particle preparations and have shown that iodination is inhibited by propylthiouracil, thiocyanate and anoxia, though perchlorate and thyrotrophin were without effect (Taurog et al, 1955). The presence of an iodide peroxidase enzyme was confirmed by Serif and Kirkwood (1958) and Alexander (1959). This enzyme has since been isolated and purified by Taurog (1970) who observed its inhibition by excess iodide in the medium, possibly explaining the Wolff-Chaikoff effect (1948). Taurog also confirmed that antithyroid

drugs reduce iodination by inhibiting the peroxidase enzyme system and localised the enzyme on apical cell membrane, a site first suggested by Benabdeylil et al (1967). Fawcett and Kirkwood (1954) postulated the existence of a tyrosine-iodinase to catalyse incorporation of iodine into tyrosyl residues of thyroglobulin, but further evidence for its existence has not been forthcoming.

Assessment of Iodide Organification In Vivo

Though still the subject of vigorous debate, most evidence is against iodide organification being a major rate-limiting factor in thyroid hormone synthesis (Berson and Yalow, 1955; Vanderlaan, 1954). Consequently, the detection of inorganic ^{131}I in thyroid indicates a defect of iodide organification.

Stanley and Astwood (1947) first demonstrated discharge of inorganic ^{131}I from glands of thiouracil-treated patients given thiocyanate and later showed perchlorate to be more potent (Stanbury and Wyngaarden, 1952). Subsequent reports of the inhibitory effect of thiocyanate on thyroidal iodide organification in vitro (Franklin et al, 1944; Alexander, 1959; Paston, 1963) and in vivo (Raben, 1949; Wollman, 1962b) have highlighted perchlorate as the most suitable discharging agent. Most workers failed to find evidence for inhibition of iodide organification by perchlorate (Halmi, 1960; Richards and Ingbar, 1959; De Groot and Buhler, 1971) though Greer (1966) elegantly demonstrated gradual inhibition of iodide organification with increasing concentrations of perchlorate in his system.

Morgans and Trotter (1957) introduced an oral perchlorate discharge test and demonstrated inorganic thyroidal ^{131}I in Hashimoto's

disease, indicating defective iodide organification in this condition. Since then, intensive study has confirmed the usefulness of this type of test though lack of sensitivity makes detection of small amounts of inorganic ^{131}I in thyroid difficult. This insensitivity is reflected in variable dosages of perchlorate, timing of its administration, and variable criteria for interpretation of the test, found among its protagonists (Fraser et al, 1960; Floyd et al, 1960; Baschieri et al, 1963; Stewart and Murray, 1966). The clinical value of this perchlorate test can be enhanced by combining the oral dose of ^{131}I with carrier iodide (Takeuchi et al, 1970; Susuki and Mashimo, 1972).

The organification rate of accumulated thyroidal ^{131}I can also be used to indicate functional integrity of iodide organification in thyroid. Using complicated kinetic analysis, Berson and Yalow (1955) failed to demonstrate inorganic thyroidal ^{131}I in hyperthyroid subjects and concluded that the organification rate was near unity. Ingbar (1955), using similar techniques, believed that free iodide was present in normal glands and computed a binding rate of 0.91 per hour in euthyroid subjects (i.e. binding of 91% of trapped iodide per hour). Since these investigators leaned heavily on both kinetic analysis and major assumptions, and since the presence or absence of free thyroidal iodide cannot easily be verified by chemical separation procedures, these reports are difficult to evaluate. Robertson et al (1971) have recently used a digital computer to calculate the iodide organification rate in vivo but these workers assumed the presence of unorganified thyroidal iodide in their analysis.

It has been reported (Owen et al, 1960) that the thyroidal ^{131}I uptake pattern found in major defects of iodide organification is one of rapid uptake followed by rapid fall due to diffusion of inorganic radionuclide from the thyroid. This feature is not shown when the defect is partial (Gray, 1973) and has little practical application.

Clinical Defects of Iodide Organification

Lerman (1946) described two hypothyroid goitrous brothers with a high thyroidal ^{131}I uptake and low thyroid content of inorganic iodine. Stanbury and Hedge (1950) later reported thiocyanate dischargeable ^{131}I in thyroid glands of three goitrous cretins and introduced the concept of thyroid dyshormonogenesis by suggesting that these observations were compatible with a congenital defect of iodide organification. Similar findings were confirmed in other hypothyroid (Stanbury, 1951; Schultz et al, 1957; Gardiner et al, 1959; McGirr et al, 1959) and euthyroid (Clayton et al, 1958) goitrous patients. Recently peroxidase enzyme deficiency has been confirmed in vitro (Valente et al, 1971; Hagen et al, 1971) in cases of familial goitre with a clinical defect of iodide organification.

Morgans and Trotter (1958) added to the spectrum of familial goitre with impaired utilisation of trapped iodide, when they found inorganic ^{131}I in the thyroid of patients with goitre-deafness syndrome, first described by Pendred (1896). Pendred's syndrome is a recessive hereditary disease (Brain, 1927; Fraser, 1964, 1965) and though in its complete form, it is characterised by congenital deafmutism, goitre and a defect in thyroidal organification of

iodide, atypical forms occur (Bax, 1966) with goitre absent (Von Harnack et al, 1961), or only slight auditory disturbance (Fraser, 1961). It is of interest that euthyroid patients with goitre and a partial defect of thyroidal iodide organification have been shown to lack peroxidase enzyme (Hagen et al, 1971) while one patient with classical Pendred's syndrome has recently been reported to have normal peroxidase activity (Lyunggren and Vecchio, 1969). This latter patient may have a different, and as yet unknown, iodinating defect.

Acquired disorders of iodide organification may be observed in the course of thyroiditis (Morgans and Trotter, 1957), following radioactive iodine therapy (Kirkland, 1954), or after drug administration. Such drugs include thionamides (Stanley and Astwood, 1947), thiocyanate (Barker, 1936), para-aminosalicylic acid (Kumrower, 1951; MacGregor and Somner, 1954), resorcinol (Bull and Fraser, 1950; Doniach and Fraser, 1950) and iodide in high dosage (Paris et al, 1960). Susceptibility to iodide inhibition is sometimes familial (Croughs et al, 1965) and is often acquired in patients treated with radioactive iodine to ameliorate hyperthyroidism (Braverman et al, 1969).

CHAPTER 2 - SUMMARY

The human and animal thyroid gland has effectively compensated for the environmental scarcity of iodide by developing an efficient ionic transport mechanism to extract iodide from plasma. This mechanism, active in nature, is stimulated by thyrotrophin (under normal physiological conditions) but it is not specific for iodide since other ions, including pertechnetate and perchlorate, may also be accumulated. Iodide transport can be shown to be biochemically distinct from iodide organification, the next step in hormone synthesis.

Following transport into thyroid follicular cells, iodide is rapidly oxidised by peroxidase and organified to tyrosyl residues in colloidal thyroglobulin. This organification reaction is specific for iodide and probably occurs when the ion traverses the apical cell membrane. There is no unanimity on whether iodide transport or organification provides the rate-limiting factor for hormone synthesis. Discharge of accumulated inorganic thyroidal ^{131}I following oral perchlorate administration currently provides the only reliable clinical index of defective iodide organification.

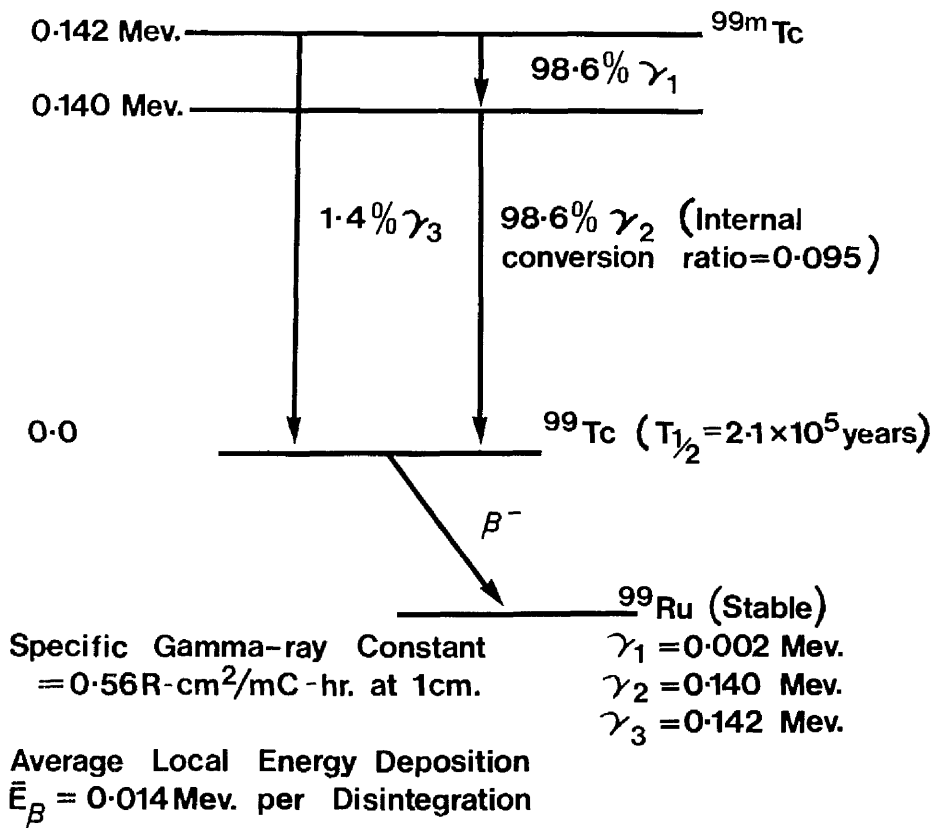


Figure 3: Decay scheme and nuclear properties of technetium-99m (^{99m}Tc) → (from Smith, 1964).

CHAPTER 3

DEVELOPMENT OF KNOWLEDGE OF TECHNETIUM

Since technetium (atomic weight 43) was first observed in stellar spectra (Harper et al, 1962; Mason, 1966) twenty-one radionuclides and metastable states of the element have been made by nuclear bombardment or nuclear fission. In 1939, Seaborg and Seagre reported the discovery of technetium-99m, metastable state of technetium-99. This radionuclide, as the peroxyanion pertechnetate, has subsequently proved of immense practical value as a biological tracer in clinical nuclear medicine.

Generation, Physical Characteristics and Dosimetry of Technetium-99m

Technetium-99m is obtained as the daughter product of molybdenum-99 (^{99}Mo) which is in turn recovered as a fission product or produced by neutron bombardment of ^{98}Mo . It is conveniently produced carrier-free as pertechnetate ($^{99\text{m}}\text{Tc}$) in isotonic saline from a ^{99}Mo generator (half-life 67 hours). Figure 3 gives the decay system and nuclear properties of $^{99\text{m}}\text{Tc}$. It has a short physical half-life of 6 hours and decays by isomeric transition emitting a 140-Kev gamma ray without primary particulate radiation. The effective E_B due to conversion electrons (8% of the 140-Kev gamma), low energy gamma and fluorescent photons, is 0.014 Mev. The specific gamma ray constant (I_γ) is 0.56 R/mc hour at 1 cm. $^{99\text{m}}\text{Tc}$ generated in the oxidised state as pertechnetate behaves

biologically like iodide, while in the reduced state (Eckelman et al, 1971) it is readily tagged to various organic compounds (Anders, 1960).

Assuming no excretion, dose calculations give 320 m rad/mc to stomach and 10 m rad total body irradiation when the radionuclide is given intravenously (Smith, 1965). ^{99}Tc arising from $^{99\text{m}}\text{Tc}$ decay gives trivial radiation exposure because of the minute amount formed (1mc $^{99\text{m}}\text{Tc}$ decays to 3.3×10^{-9} mCi ^{99}Tc) and its long half-life (2×10^5 years).

Distribution of Pertechmetate

Harper et al (1962) demonstrated a similarity in body distribution of pertechmetate and inorganic iodide. Confirmation and extension of these studies by Sorenson and Archambault (1963) and Andros (1965) revealed selective concentration of pertechmetate by salivary glands, stomach and thyroid, but exclusion from CSF. In contrast to the predominant urinary excretion of iodide (Skanse, 1948), Andros reported that only 30% of administered pertechmetate was recovered from urine in 24 hours while its cumulative faecal excretion at 72 hours was 20 - 30%. Using whole body counting to measure $^{95\text{m}}\text{Tc}$ (60 day half-life) and ^{96}Tc (4.3 day half-life), Beasley et al (1966) confirmed more precisely an initial rapid urinary excretion of pertechmetate and subsequent slower faecal excretion. Bio-transformation of pertechmetate to other chemical forms in liver with subsequent biliary excretion (Abdel-Wahab et al, 1967) is a plausible explanation for the pattern of faecal excretion.

Thyroid Physiology of Pertechmetate

Technetium is a member of group VII A of the periodic table. As pertechmetate, it is actively concentrated by the thyroid along with other members of the VII TH group namely iodide, bromide, astatide and perrhenate (Wolff, 1964). Wolff (1962) assumed that thyroid transport of these anions was enzyme catalysed and found that the T/S (pertechmetate) was greater than T/S (iodide) in sheep thyroid slides when anion concentrations were adjusted to the Km (T/s being the ratio of anion uptake per g thyroid to plasma anion concentration per ml). He calculated Km values (half-saturation) of $3-5 \times 10^{-7} \text{m}$ for pertechmetate and $3 \times 10^{-5} \text{m}$ for iodide. Wolff (1963) further demonstrated that, like iodide transport, pertechmetate transport required cellular integrity, metabolic energy and K^+ , and in addition, that pertechmetate, iodide and perchlorate could each inhibit thyroid transport of the other two anions with a potency determined by the individual Km value. He concluded that pertechmetate and perchlorate were competitive inhibitors of iodide transport and that these ions share with iodide, the same transport mechanism. As previously described, their kinship appears due to a basic similarity in molecular size, shape and charge (Wolff, 1964).

While it is accepted that iodide is the only halide which undergoes further metabolism in thyroid, there is debate about binding of pertechmetate to thyroid tissue. In well conceived studies on patients (Andros et al, 1965; Shimmins et al, 1969b) and rats (Heck et al, 1968), no binding of pertechmetate to thyroid protein was

detected. Other in vitro studies using rat thyroid (Socolow and Ingbar, 1967; Papadopoulos et al, 1967) and in vivo clinical studies (Burke et al, 1972) have reported the presence of minimal pertechnetate binding. Using chromatography, Abel-Wahab et al (1967) have detected small amounts of pertechnetate labelled iodotyrosines in urine. Clearly, the weight of evidence suggests that a small though variable portion of transported pertechnetate does undergo chemical combination with a thyroid protein.

The thyroid unidirectional clearance of pertechnetate is approximately half the iodide unidirectional clearance in vitro (Andros, 1971) despite the lower pertechnetate K_m (Vide supra). This is possibly due to plasma protein binding of the carrier-free pertechnetate used in these studies (Wolff and Maurey, 1962; Oldendorf et al, 1969; Hays and Green, 1971) thereby reducing the availability of plasma pertechnetate to the thyroid transport mechanism.

Role of Pertechnetate in Thyroid Investigation

In the last decade, ^{99m}Tc has become one of the most widely used radionuclides in clinical nuclear medicine. Its physical characteristics which epitomise the ideal radioactive tracer (Herbert et al, 1965) include a low gamma energy permitting easy collimation without excessive tissue absorption, minimal β emission, short half-life allowing daily tests, and its carrier-free availability. A comparison between the physical properties of radionuclides used in investigation of thyroid disorders is shown

TABLE 1

PRINCIPAL PHYSICAL PROPERTIES OF RADIOACTIVE ISOTOPES

USED IN THE INVESTIGATION OF THYROID DISORDERS

	HALE-LIFE	AVERAGE B EMISSION	EQUIVALENT B EMISSION DUE TO X-RAYS	K FACTOR (γ) (R.cm ² /mCi.h)	K FACTOR (X-RAY) R.cm ² /mCi.h	PREDOMINANT X EMISSION
¹²³ I	13 hours	0	31 KeV	0.646	0.816	160 KeV
¹²⁵ I	57 days	0	21 KeV	0	1.28	27 KeV
¹³¹ I	8.05 days	190 KeV	negligible	2.25	negligible	364 KeV
¹³² I	2.3 hours	490 KeV	negligible	11.8	negligible	670,780 KeV
^{99m} Tc	6 hours	0	14 KeV	0.56	0.16	140 KeV

From Goolden et al, 1968.

TABLE 2

RADIATION DOSE TO ADULT THYROID (Rads/ μ Ci) FROM
RADIONUCLIDES USED FOR THYROID INVESTIGATION

^{131}I	1520×10^{-3}
^{132}I	17×10^{-3}
^{125}I	1120×10^{-3}
^{123}I	16×10^{-3}
$^{99\text{m}}\text{Tc}$	0.2×10^{-3}

From Goolden et al, 1968

in Table 1. The radiation exposure to the thyroid from each radionuclide is shown in Table 2.

A 15 - 20 minute thyroid uptake of ^{99m}Tc has been measured using directional scintillation counting (Andros et al, 1965; Van't Hoff et al, 1972), quantitative scintiscanning (Andros et al, 1965; Shimmins et al, 1968b; Atkins and Richards, 1968; De Garretta et al, 1968) and scintillation camera techniques (Hurley et al, 1972; Burke et al, 1972). The range of ^{99m}Tc uptake reported for subjects with normal thyroid function was 0.5 - 3.0% administered dose, while in patients with thyrotoxicosis it was 5 - 40% dose. In subjects with simple goitre the range was 1 - 5% dose, and in patients with primary hypothyroidism, less than 1% dose. Hauser (1971) noted that the uptake in Hashimoto's disease tended to be higher than normal with a range of 1 - 8% dose. Although the diagnostic information obtained from measurement of ^{99m}Tc uptakes is readily available from equivalent radioactive iodine uptake parameters (McGill et al, 1971; Gray et al, 1973), use of ^{99m}Tc does confer some distinct advantages to both clinician and patient. Firstly, the low radiation exposure to thyroid from ^{99m}Tc allows less hazardous thyroid studies on children (Dodds and Powel, 1967) and pregnant women. Secondly, as ^{99m}Tc kinetics are uninfluenced by concurrent administration of drugs which block thyroidal iodide organification, the ^{99m}Tc uptake can be used as an index of hyperthyroid disease activity in patients taking antithyroid medication (Goolden et al, 1971) and can also indicate thyroid

suppressibility (Shimmins et al, 1971; Goolden et al, 1971).

Using quantitative scintiscanning, Shimmins et al (1968b) measured thyroid unidirectional clearance of ^{99m}Tc and found a mean clearance in hyperthyroid patients of 287 ml/min compared to 37 ml/min in normal subjects. Lack of confirmatory data in the literature reflects the complexity of the kinetic analysis required for this measurement.

CHAPTER 3 - SUMMARY

Although pertechnetate- ^{99m}Tc (^{99m}Tc) has an 'iodide-like' distribution in body tissues with concentration in thyroid, salivary glands, gastric mucosa and choroid plexus, its binding to plasma proteins and predominant faecal excretion are in direct contrast to iodide physiology. ^{99m}Tc shares the thyroïdal iodide transport mechanism with perchlorate and other ions because they are similar to iodide in molecular size and shape. Organification of thyroïdal ^{99m}Tc to tyrosyl residues in follicular colloid does not occur to any major extent. While thyroid uptake studies with ^{99m}Tc provide the same fundamental information as early uptake studies with ^{131}I , advantages of ^{99m}Tc include a low radiation exposure to patient and attendant personnel and a facility for measurement during antithyroid drug therapy.

CHAPTER 4

SIMULATION OF THYROIDAL IODIDE METABOLISM

When Borelli (1608-1679), Professor of Mathematics at Pisa, used his knowledge of mechanics to simulate the human skeletal system, he initiated a technology which has stimulated the imagination of successive generations of medical scientists and bioengineers (Lenihan, 1972). A recent expansion in hospital computer services has generated renewed interest in mathematical simulation and its applications to the field of medicine (Pack and Murray-Smith, 1973).

Introduction of radioactivity increased the scope of biological simulation (Robertson, 1957) and studies of tracer behaviour in living organisms (kinetic analysis) has allowed a dynamic portrayal of complex metabolic events, unobtainable within the confines of more conventional disciplines such as biochemistry.

Compartmental Analysis in Radioactive Tracer Studies

For mathematical purposes, constituents of living systems must be regarded as being located in distinguishable phases or volumes of homogeneous composition which are delineated physically or chemically but not necessarily anatomically (Sheppard and Householder, 1951). These are designated pools or compartments. The rate-constant of transfer of a substance from one compartment to another is that fraction of the compartment which enters another in unit time (i.e. the clearance of one compartment into another). A biological model

is constructed by defining the compartments and routes of transfer between compartments (Stanbury and Brownell, 1954). In radioactive tracer studies, models are fortunately represented mathematically by simple linear differential equations.

Basic assumptions critical to this type of analysis have been outlined by Matthews (1971). These include firstly an assumption that there is uniform distribution of substance in each compartment at all times with instantaneous and homogeneous mixing of tracer with tracee. Secondly, that behaviour of the tracer is identical to that of tracee in the compartment. Despite the doubtful validity of the first assumption, model theory (Brownell et al, 1968) and practice have played a valuable role in clinical medicine, notably in the thyroid arena (Brownell, 1951; Berman, 1968; De Groot et al, 1971a; Alexander et al, 1971) but also in other fields (I.A.E.A., 1971).

In common with other advancing technologies, compartmental analysis will be seen to be a phase in the development of radionuclide kinetic analysis. A second phase has possibly already begun with the concept of occupancy (Orr and Gillespie, 1968).

Mathematical Models of Thyroidal Iodide Transport and Organification

Although the precise mechanisms of iodide transport and organification are unknown, mathematical models have been devised which agree well with experimental data (Wolff, 1964). Useful information has been obtained in man (Berson and Yalow, 1955) and in animals (Wollman, 1954; Wollman and Reed, 1959; Wollman and Reed,

TWO COMPARTMENT THYROID MODEL .

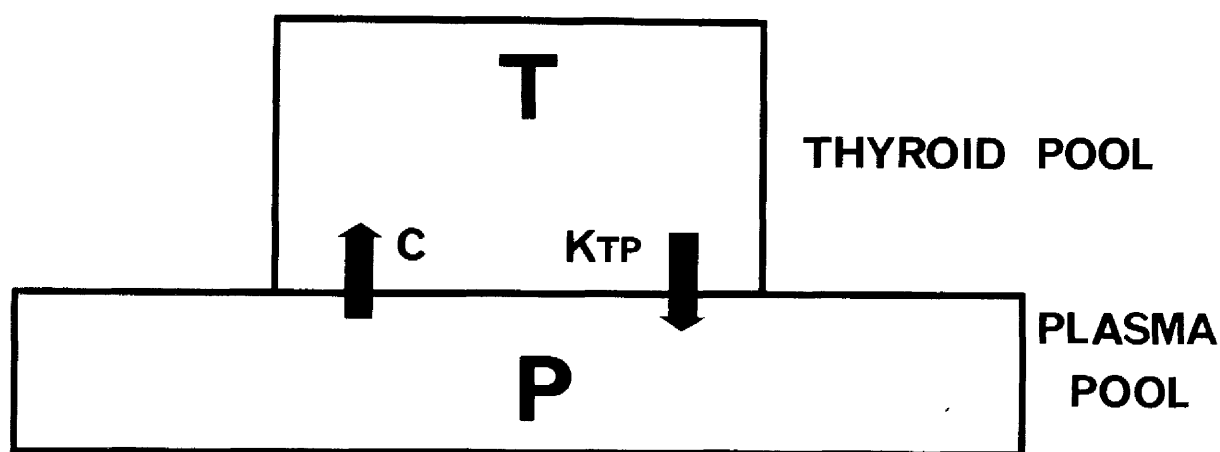


Figure 4: Two-compartment thyroid model where T is the fraction of administered radionuclide (^{131}I or $^{99\text{m}}\text{Tc}$) in the thyroid pool, P fraction/Litre is the plasma concentration of radionuclide, C Litres/min is the thyroïdal unidirectional clearance and $K_{TP} \text{ min}^{-1}$ the thyroid exit-rate constant of radionuclide.

1962a) using kinetic analysis of the equilibration curves of blood and thyroidal radioactive iodine. It must be stressed, however, that with major assumptions involved, rate-constant values obtained represent informed approximation rather than scientific accuracy (Bergner, 1962). Model analysis has been performed graphically (Shimmins et al, 1968b), by analogue (Brownell, 1951; Hickey and Brownell, 1954; Rollinson and Rotblat, 1955) and by digital computation (Robertson et al, 1971).

Two Compartment Thyroid Model

When organification of iodide in thyroid is blocked with a thiocarbamide drug, radioactive iodide (^{131}I) concentration by thyroid is the resultant of two processes, namely ^{131}I transport to thyroid from plasma and ^{131}I diffusion from thyroid to plasma. A similar concept is appropriate for pertechnetate which is unbound in thyroid tissue.

In a two compartment model (Figure 4), the thyroid is considered to be a structureless compartment containing a uniform concentration 'T' of ^{131}I . Exchange of plasma ^{131}I at concentration 'P' with thyroidal ^{131}I is characterised by the linear differential equation.

$$dT/dt = C.P. - K_{TP} \cdot T.$$

Thyroid unidirectional clearance of ^{131}I (C) equals $V \times F$ where V is thyroid plasma flow and F is the fraction of plasma ^{131}I transferred from plasma to thyroid. Thyroid exit rate-constant, ' K_{TP} ', is the fraction of thyroidal ^{131}I transferred to plasma in unit time.

THREE COMPARTMENT THYROID MODEL.

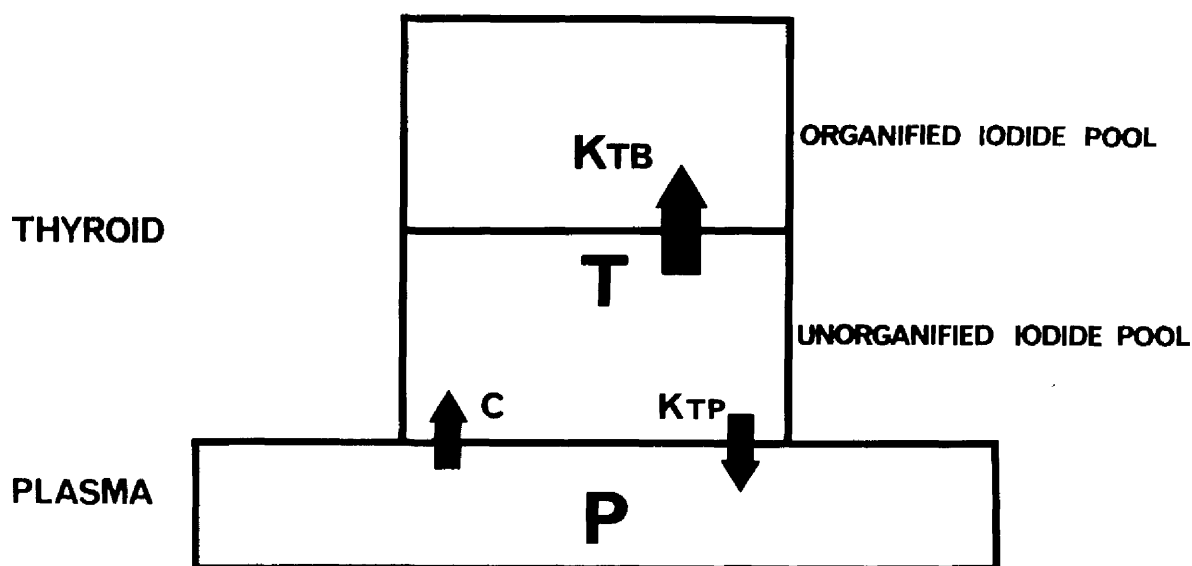


Figure 5: Three-compartment thyroid model where T is the fraction of administered ^{131}I in the thyroidal inorganic iodide pool, P fraction/Litre is the plasma concentration of inorganic ^{131}I , C Litres/min is the thyroidal unidirectional clearance (of ^{131}I) $k_{TP} \text{ min}^{-1}$ the exit-rate constant and $k_{TB} \text{ min}^{-1}$ the organification-rate constant of the thyroidal inorganic ^{131}I pool.

Wollman and Reed (1959) have shown mathematically that the shape of thyroid equilibration curves is determined by the time dependence of plasma ^{131}I concentration and by the exit rate-constant.

Three Compartment Thyroid Model

When iodide organification is permitted, simulation is possible by adding an organic iodine compartment to the basic two compartment model (Figure 5). In this model, the thyroid is considered to be two structureless compartments, one containing a uniform concentration 'T' of inorganic ^{131}I , the other a uniform concentration 'B' of organified ^{131}I . Plasma ^{131}I at concentration P is transferred to the thyroidal inorganic ^{131}I compartment at a rate equal to the ^{131}I content of C litres of plasma/min. Of thyroidal ^{131}I (T), a constant fraction equal to ' K_{TB} ' is incorporated into the bound compartment each minute. A second fraction, K_{TP} diffuses from thyroid to plasma each minute. The complete process is characterised by the linear differential equation.

$$dT/dt = CP - K_{\text{TP}}T - K_{\text{TB}}T.$$

It must be assumed that compartmental concentrations of stable iodide are constant and that no organified ^{131}I is metabolized during the period of study.

Workers using the three compartment model have predicted inorganic ^{131}I in normal human thyroid glands (Ingbar, 1955) and in animals (Wollman and Reed, 1962a), while others feel that there is insufficient evidence for this prediction in humans (Berson and Yalow, 1955). As each study leans heavily on either kinetic analysis with its inherent

assumptions, or chemical separation of inorganic ^{131}I from excised thyroid tissue, it is currently accepted (Rall et al, 1964; De Groot, 1965) that until fresh evidence is available, the presence of ^{human} inorganic iodide in/thyroid under normal binding conditions must remain sub judice. Despite limitations imposed by ignorance of thyroidal iodide pools, the author found the concept of a three compartment model useful and particularly suited to measurement of the ^{131}I organification rate K_{TB} .

SECTION 2

TECHNICAL ASPECTS OF THYROIDAL KINETIC STUDIES

IN VIVO

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| Chapter 5 | The extrathyroidal neck radioactivity |
| Chapter 6 | Arterio-venous difference: a systematic error
of thyroid clearance measurement |

CHAPTER 5

THE EXTRATHYROIDAL NECK RADIOACTIVITY

This chapter describes the development and assessment of a special collimator, designed to reduce the contribution of extrathyroidal neck radioactivity to total neck counts during thyroid uptake measurements.

INTRODUCTION

It is difficult to measure with precision the quantity of radionuclide (usually ^{131}I or $^{99\text{m}}\text{Tc}$) in thyroid. Although allowance for scattered radiation can be made using a thyroid phantom to approximate in vivo conditions, systematic errors due to variation in position and anatomy of the gland cannot be entirely eliminated.

In addition to thyroid radioactivity, a counter placed over the neck will detect radionuclide present in plasma, extracellular fluid and salivary glands. This extrathyroidal activity (E.T.A.) is usually 5 - 7% of administered ^{131}I dose when the I.A.E.A. collimator is used (Hilditch et al, 1967; Andros et al, 1965). Since a normal thyroid uptake of ^{131}I or $^{99\text{m}}\text{Tc}$ 20 minutes after injection is only 1 - 4% of administered dose, uncertainty in estimating the correction for other neck radioactivity leads to unacceptably large errors in the derived thyroid uptake (Shimmins et al, 1968a).

Various methods have been proposed for the indirect estimation

of E.T.A. with precision in thyroid uptake measurement the prime objective. Some authors have determined E.T.A. by counting over the neck with a thick lead shield covering the thyroid (Floyd et al, 1960; Alexander et al, 1962) while others have used the counting rate over the thigh (Myant et al, 1949a; Goolden and Mallard, 1958; Fraser et al, 1960). In contrast, various workers calculated E.T.A. by making assumptions regarding its variation with time. Berson et al (1952) assumed that E.T.A. was a constant fraction of ^{131}I not trapped by the thyroid or excreted by the kidney, while Veall and Vetter (1958) and Koutras and Sfontouris (1966) assumed that E.T.A. changed in proportion to the plasma ^{131}I radioactivity. Oddie et al (1955), made no assumptions and found that when E.T.A. was expressed as a fraction of ^{131}I radioactivity remaining in the body and not in the thyroid, it was not constant but did not fall as rapidly as plasma radioactivity. He recommended the routine use of a mean value of E.T.A. obtained in a group of patients using standard neck counting geometry. Hilditch et al (1967) recently confirmed Oddie's findings using quantitative scintiscanning techniques to selectively measure the contribution of E.T.A. to total neck radioactivity. In similar studies Shimmins et al have formulated equations for calculating the early thyroid uptake of ^{131}I (1968a) and $^{99\text{m}}\text{Tc}$ (1971). These equations also depend on the mean value of E.T.A. obtained in a group of patients.

Since a high absolute value of E.T.A. is inappropriate for precise thyroid kinetic analysis, the author reduced the volume of

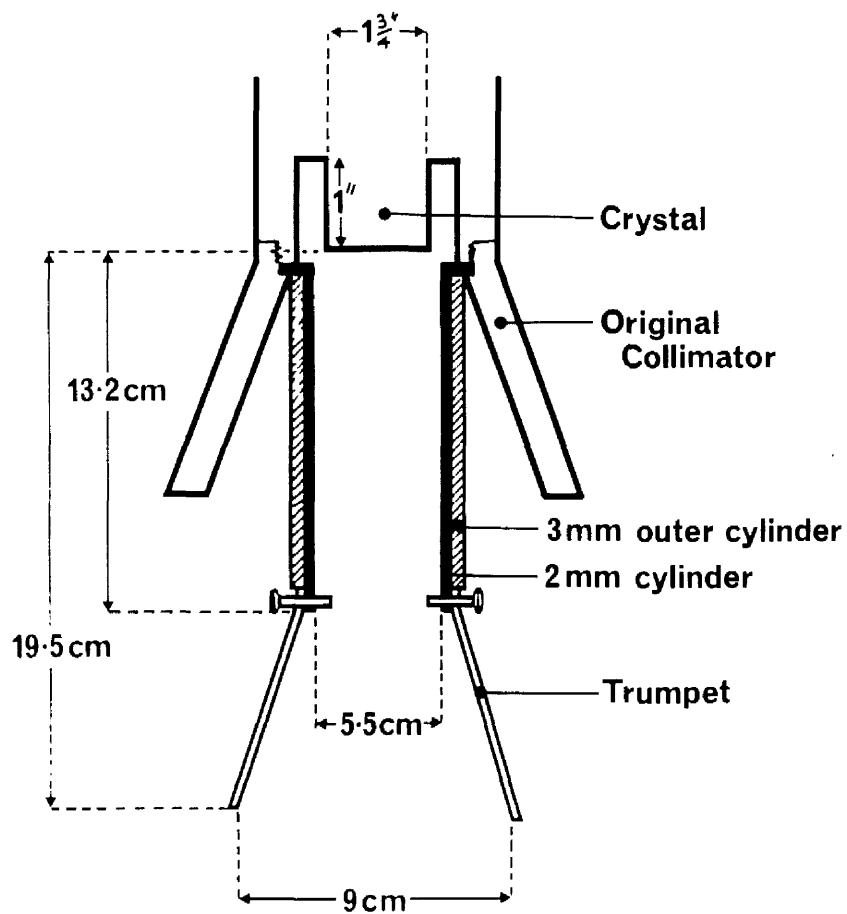


Figure 6: Diagram of special collimation showing original collimator and addition in long axis.

neck 'seen' by the counter by constructing a simple collimator which still included the whole of thyroid in its field. The physical characteristics of this collimator were evaluated and accuracy of E.T.A. measurement was assessed.

MATERIALS AND METHODS

(a) Collimation

The collimator was used with a directional scintillation counter (I.D.L. type 6630). Review of 300 routine rectilinear thyroid scintiscans taken over the preceding twelve months with a Selo scanner showed that 94% of all thyroid images could be included in a 7.5 x 7.5 cm rectangle. The aim of collimator design was therefore to view an area of maximum response of at least 7.5 x 7.5 cm. The final system (Figure 6) consisted of a sodium iodide crystal detector ($1\frac{3}{4}$ " diameter x 1" thick) mounted within the original diverging collimator which extended $3\frac{1}{2}$ " beyond the surface of the crystal where it presented a $3\frac{1}{2}$ " diameter circular viewing area. To this collimator was added an axial cylinder of 2 mm lead with internal diameter 5.5 cm and extent 13.2 cm beyond the crystal. A trumpet of 2 mm lead, added to the end of the cylinder, was of rectangular cross-section (9 x 6 cm) at its outer edge but tapered to fit the cylinder at the other end. Overall length of additional material was 18.8 cm (19.5 cm from the crystal) and the intended collimator to skin distance was 2 cm. Finally, a second lead cylinder 3 mm thick was placed around the first.

(b) Collimator Characteristics

The collimator response and field of view were measured using

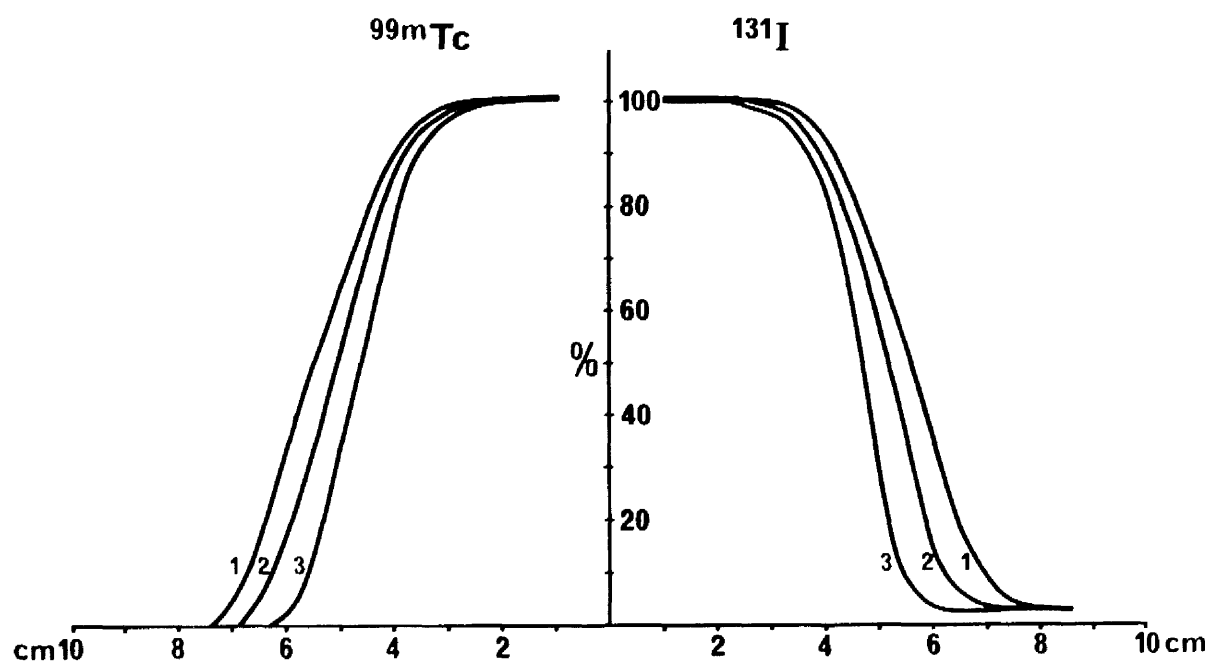


Figure 7: Visual field of special collimator for point sources of ^{131}I and ^{99m}Tc in air moved along the long axis at distances of 25.5 cm (1), 23.5 cm (2) and 21.5 cm (3) from the crystal.

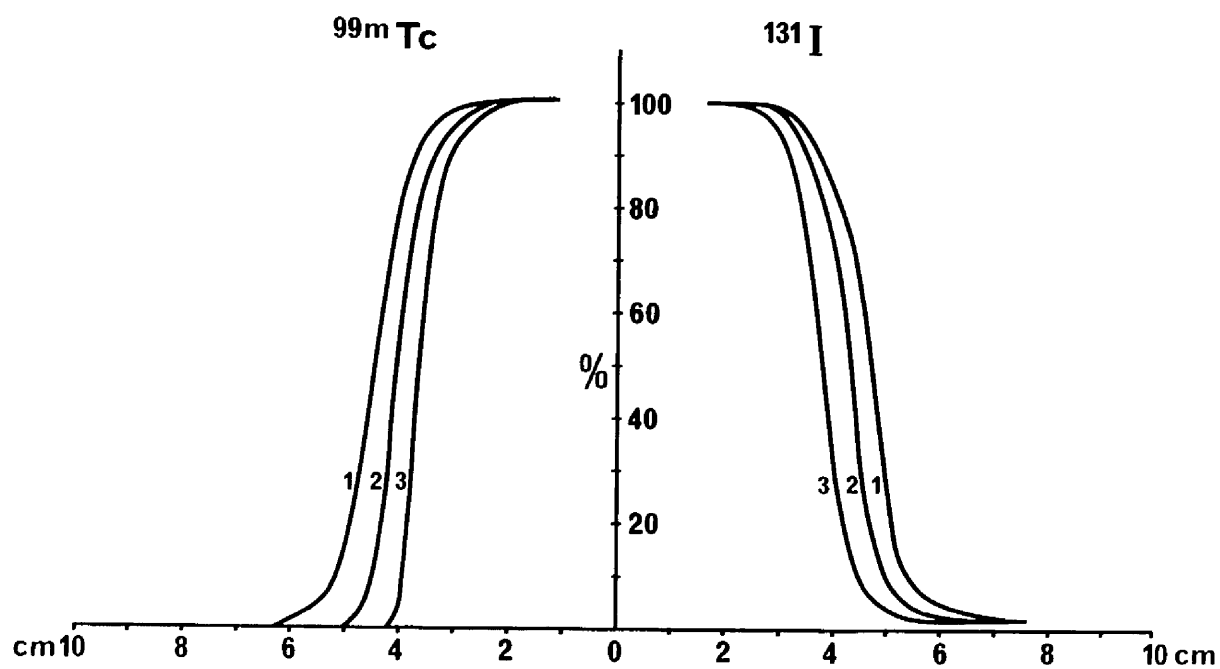


Figure 8: Visual field of special collimator for point sources of ^{131}I and ^{99m}Tc in air moved along the short axis at distances of 25.5 cm (1), 23.5 cm (2) and 21.5 cm (3) from the crystal.

small sources of ^{131}I (360 keV) and $^{99\text{m}}\text{Tc}$ (140 keV) both for long and short axis of the trumpet at distances of 21.5, 23.5 and 25.5 cm from the crystal.

(c) Extrathyroidal Radioactivity Study

E.T.A. of ^{131}I and $^{99\text{m}}\text{Tc}$ were measured following their separate and simultaneous intravenous injection (25 and 400 uCi respectively) in normal subjects whose thyroid uptake had been completely blocked with oral potassium perchlorate. A ratemeter and potentiometric recorder were used for each radionuclide giving a continuous recording of E.T.A. Radionuclide standards in a thyroid phantom were used for system calibration.

Random Errors

1) Statistical variation in output voltage of ratemeter

Error for ^{131}I was approximately 1% and for $^{99\text{m}}\text{Tc}$ less than 1%.

2) Repositioning of patients

With a reproducibility of collimator - neck distance of ± 0.5 cm, both ^{131}I and $^{99\text{m}}\text{Tc}$ had an error of approximately 5%.

3) Injection calibration

Standards had an approximate error of 3%.

RESULTS

(a) Collimator Characteristics

The collimator response curves in the long (Figure 7) and short axis (Figure 8) were virtually identical for both ^{131}I and $^{99\text{m}}\text{Tc}$. Although not ideal in

TABLE 3

MEAN E.T.A. OF ^{99m}Tc AND ^{131}I AT 5, 10, 15 AND 20 MINUTES
AFTER THEIR SINGLE AND SIMULTANEOUS INJECTION AS % DOSE \pm 2SD

NUMBER OF PATIENTS	ISOTOPE GIVEN	5				10				15				20			
		^{99m}Tc	^{131}I	^{99m}Tc	^{131}I	^{99m}Tc	^{131}I	^{99m}Tc	^{131}I	^{99m}Tc	^{131}I	^{99m}Tc	^{131}I	^{99m}Tc	^{131}I	^{99m}Tc	^{131}I
5	^{99m}Tc	1.79 ± 0.58	-	1.71 ± 0.59	-	1.66 ± 0.51	-	1.63 ± 0.50	-	-	-	-	-	-	-	-	-
5	^{131}I	-	1.96 ± 0.64	-	1.93 ± 0.63	-	1.89 ± 0.61	-	1.89 ± 0.61	-	1.89 ± 0.61	-	1.89 ± 0.61	-	1.89 ± 0.61	-	1.89 ± 0.61
5	$^{99m}\text{Tc} + ^{131}\text{I}$	1.99 ± 0.50	1.87 ± 0.61	1.90 ± 0.53	1.82 ± 0.55	1.80 ± 0.48	1.78 ± 0.55	1.74 ± 0.45	1.78 ± 0.55	1.74 ± 0.45	1.75 ± 0.50	1.74 ± 0.45	1.75 ± 0.50	1.74 ± 0.45	1.75 ± 0.50	1.74 ± 0.45	1.75 ± 0.50

sharpness of cut off at greatest distances from the central axis, the collimator gave a 90% response within a maximum area of 8 x 7.5 cm at a working distance of 25.5 cm from the crystal.

- (b) Extrathyroidal Radioactivity Study The results are shown in Table 3. Each E.T.A. value given is the mean of three of measurements at intervals of 24 hours on each/five patients, a total of fifteen measurements.

It can be seen that

- 1) E.T.A. is approximately 1 - 2% of administered dose for both ^{131}I and $^{99\text{m}}\text{Tc}$.
- 2) There is a close correlation between the E.T.A. of ^{131}I and $^{99\text{m}}\text{Tc}$ when measured simultaneously.
- 3) The difference between the E.T.A. at 5 and 20 minutes is small (i.e. approximately an 8% fall).

The error (± 2 S.D.) for E.T.A. measurement in a single subject was approximately 10% for $^{99\text{m}}\text{Tc}$ and 20% for ^{131}I .

DISCUSSION

In previous thyroid ^{131}I uptake and clearance studies using directional counting, measurement precision was precluded both by the high absolute value of E.T.A. and by errors inherent in its indirect estimation. Early attempts to measure thyroid uptake of $^{99\text{m}}\text{Tc}$ with similar systems were likewise abandoned because of the E.T.A. problem (Andros et al, 1965). Though technological advance in the form of quantitative scintiscanning (Hilditch et al, 1967)

and scintillation camera techniques (Hurley et al, 1972) have obviated this particular source of error, the expense and technical knowledge required for these studies limits the facility to major centres.

With the closer collimation described, the author reduced E.T.A. to 30% of the value obtained with an I.A.E.A. collimator. Furthermore, as individual measurements of E.T.A. were acceptably reproducible, the error in early thyroid uptake measurement was greatly reduced from approximately 40% to 10%. It is interesting that E.T.A. of both radionuclides is similar because, despite identical collimator fields for ^{99m}Tc and ^{131}I , the lower energy gamma ray of ^{99m}Tc is more easily absorbed in neck tissues. This finding might indicate that most of E.T.A. is anteriorly placed and not uniformly distributed.

The prescribed reduction in collimator field does impose a restriction on the size of goitre for which accurate measurements are possible. The author has found that an 8 x 7.5 cm rectangle (90% response) is adequate to cover all but very large thyroids. In only 6% of 300 routine thyroid scintiscans was thyroid greater in size than this and the majority of these were large non-toxic goitres of > 100 g.

This collimator has been used for all subsequent in vivo human studies reported in this thesis.

SUMMARY - CHAPTER 5

A closely collimated scintillation detector was constructed specifically to reduce the contribution made by extrathyroidal radioactivity to total neck radioactivity during measurements of the uptake of ^{131}I and $^{99\text{m}}\text{Tc}$ by the thyroid. The error of these measurements was thereby reduced from 40% to 10%. The restricted field of the collimator was found to be adequate for uptake measurements in all thyroid glands under 100 g in weight.

CHAPTER 6

ARTERIO-VEINUS DIFFERENCE : A SYSTEMATIC ERROR OF THYROID CLEARANCE MEASUREMENT

This chapter describes the detection of a significant difference between arterial and venous plasma concentrations of radioactive iodine and pertechnetate- ^{99m}Tc at short time intervals after their intravenous injection. The resulting systematic error in measurement of thyroidal radioactive iodine clearance had not been previously recognised. A study of measures designed to reduce this error is also reported.

INTRODUCTION

The thyroid clearance of radioactive iodide- ^{131}I after intravenous injection is known to be higher in the first few minutes after administration than at later times (Schultz and Zieve, 1957; Koutras and Sfontouris, 1966; Yamamoto et al, 1969). Explaining this phenomenon, Rall and co-authors (1964) described normal thyroid in terms of an open three-compartment model and distinguished between 'unidirectional' and 'net' clearance rates. The 'unidirectional' clearance corresponds to the initial flux of ^{131}I ions from plasma to the hypothetical iodide pool in thyroid while 'net' clearance represents a balance between ^{131}I ions entering and leaving the thyroidal iodide pool at later times.

Though Rall's explanation is plausible, the author's failure to detect free intrathyroidal iodide in normal and thyrotoxic subjects

(Gray et al, 1973) was incompatible with this theory. It seemed appropriate therefore to investigate possible sources of systematic error which could have resulted in an apparent fall in thyroid clearance of ^{131}I at early intervals after injection. Two possible sources of error were studied. Firstly, a slow diffusion of plasma ^{131}I into red cells could progressively reduce plasma ^{131}I concentration in venous blood samples awaiting radioactivity counting. Confirmation (Gray et al, 1973) of the rapid equilibration between plasma and red cell ^{131}I first described by Myant et al (1950) excluded this possibility. A second possible source of error was slow mixing of injected ^{131}I between arterial and venous plasma pools which, with venous sampling, would result in underestimation of ^{131}I concentration in arterial plasma perfusing thyroid. Accordingly, a study was made of the arterio-venous difference of ^{131}I and pertechnetate- $^{99\text{m}}\text{Tc}$ ($^{99\text{m}}\text{Tc}$) in euthyroid and thyrotoxic patients following radionuclide injection. In addition, the precision of praecordial radioactivity counting for paralleling arterial plasma radioactivity was studied as an alternative to arterial plasma sampling.

MATERIALS AND METHODS

Six male patients were studied initially and all gave their consent after careful explanation of the procedure. Four patients were euthyroid and had a 4 inch teflon arterial catheter in the (R) brachial artery for respiratory investigation. None was in respiratory failure. Two untreated thyrotoxic patients each had a

(R) brachial arterial catheter inserted by an experienced operator. Following an intravenous injection of 25 uCi ^{131}I and 400 uCi $^{99\text{m}}\text{Tc}$ to each subject in the (R) arm, simultaneous arterial and venous blood samples were taken at intervals from 1 to 20 minutes following radionuclide administration. Venous samples were taken from a small venous cannula in the (L) arm. Plasma samples (2 ml) were counted for ^{131}I and $^{99\text{m}}\text{Tc}$ in a well-type scintillation counter.

The thyroid uptake curve of ^{131}I was followed in the two thyrotoxic patients by a directional counting and recording system previously described (Chapter 5). Extrathyroidal radioactivity of ^{131}I was subsequently measured in each patient by repeating the 25 uCi ^{131}I dose 5 minutes after an intravenous injection of 100 mg sodium perchlorate. Increased radioactivity in the system was considered extrathyroidal and subtraction from the initial thyroid uptake curve allowed quantitation of the rate of ^{131}I uptake by thyroid.

In a further 4 euthyroid patients, arterial and venous plasma radioactivities were measured following a 30 second intravenous infusion of 400 uCi $^{99\text{m}}\text{Tc}$. In addition, however, the praecordial radioactivity curve was monitored by a similar counting and recording system to that used for thyroid uptake studies. Calibration of the praecordial count rate was obtained by equating the 20 minute venous and praecordial radioactivities. An infusion technique was used since radionuclide injection resulted in a variable praecordial count rate in the first minutes after administration, possibly due to a bolus effect.

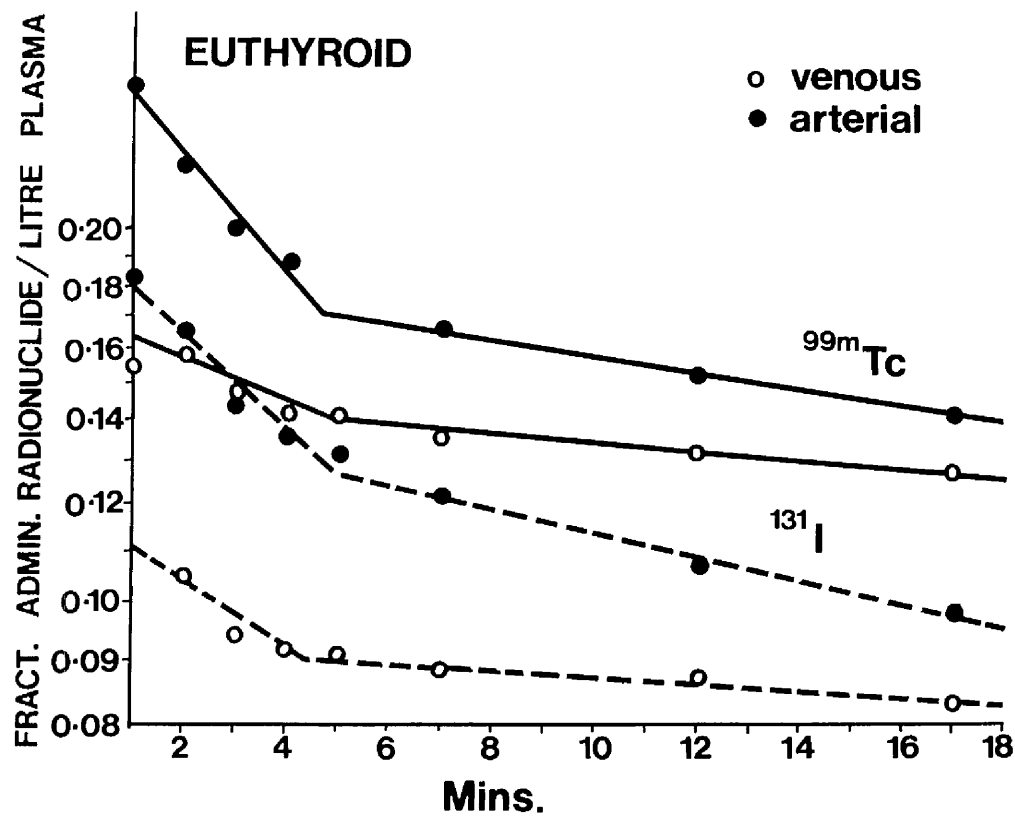


Figure 9: Arterial and venous plasma concentrations of ^{131}I and $^{99\text{m}}\text{Tc}$ at early intervals following their simultaneous intravenous injection in a euthyroid subject.

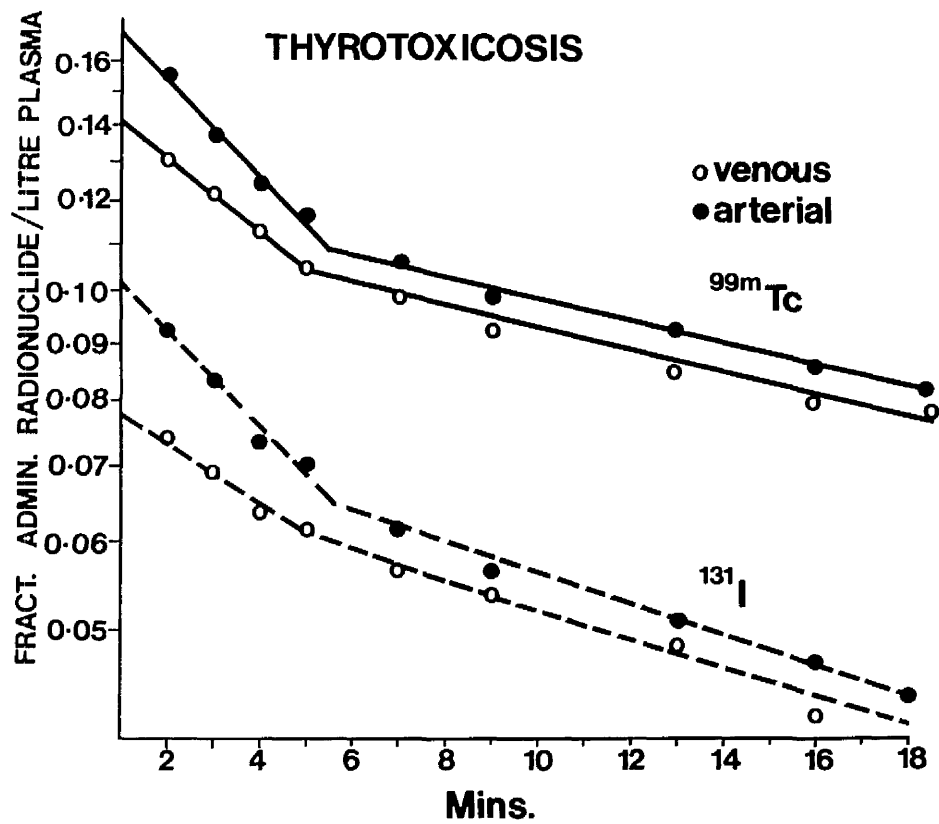


Figure 10: Arterial and venous plasma concentrations of ^{131}I and $^{99\text{m}}\text{Tc}$ at early intervals following their simultaneous intravenous injection in a thyrotoxic subject.

TABLE 4

THYROIDAL ^{131}I NET CLEARANCE, MEASURED USING ARTERIAL AND VENOUS
PLASMA RADIOACTIVITIES AT INTERVALS AFTER IV RADIONUCLIDE INJECTION

<u>MINUTES POST INJECTION</u>	<u>THYROIDAL ^{131}I CLEARANCE (ml/min)</u>			
	<u>SUBJECT 1</u>		<u>SUBJECT 2</u>	
	<u>ARTERIAL</u>	<u>VENOUS</u>	<u>ARTERIAL</u>	<u>VENOUS</u>
2 - 6	73	88	81	97
6 - 10	68	71	80	88
10 - 22	68	70	71	71

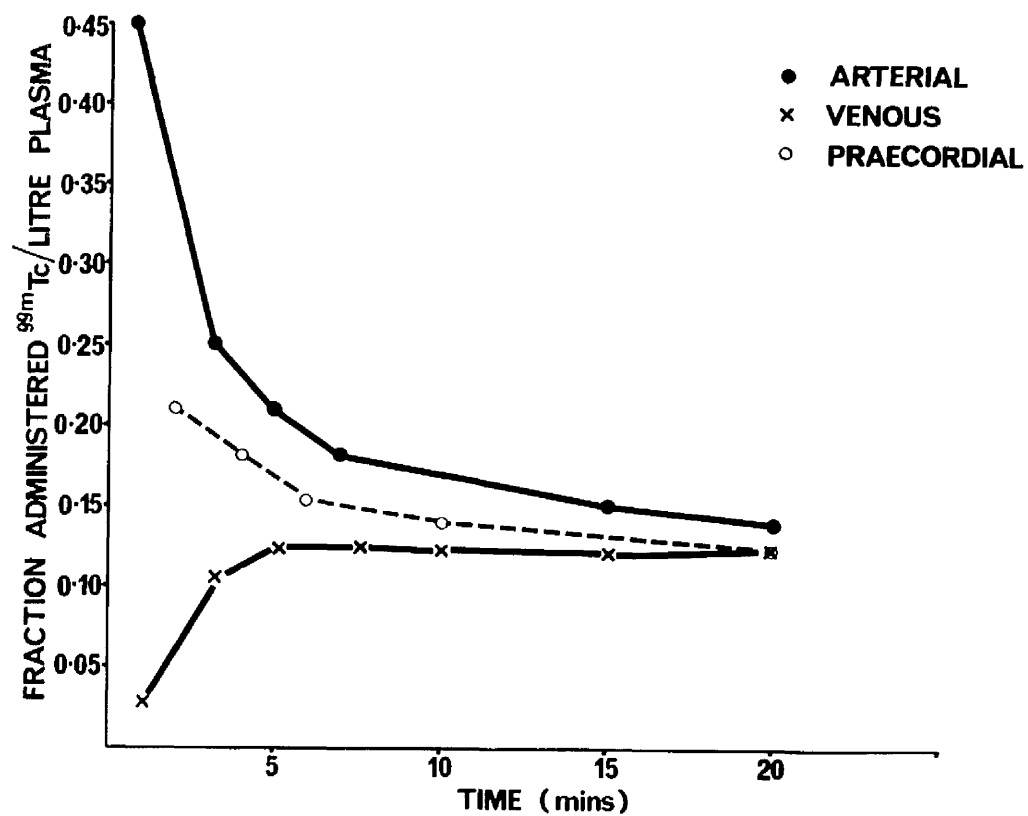


Figure 11: Arterial plasma, venous plasma and praecordial radioactivities following a 30 second intravenous infusion of ^{99m}Tc . By equating the praecordial and venous radioactivities at 20 minutes, quantitative extrapolation of earlier praecordial radioactivity is possible.

RESULTS

Decay curves of arterial and venous plasma ^{131}I and $^{99\text{m}}\text{Tc}$ radioactivities in a euthyroid subject are shown in Figure 9. Corresponding ^{131}I and $^{99\text{m}}\text{Tc}$ plasma curves from a thyrotoxic subject are shown in Figure 10. Similar curves were found in all other subjects studied. The notable feature is a significant difference between arterial and venous concentration of radionuclide in the first 6 minutes after injection. Thereafter, arterio-venous difference is less pronounced. Although decay rates of plasma ^{131}I and $^{99\text{m}}\text{Tc}$ are similar, there is a persistently higher concentration of $^{99\text{m}}\text{Tc}$.

Measurements of thyroid ^{131}I clearance in the two thyrotoxic patients at intervals following radionuclide injection are shown in Table 4. The calculation has been made using arterial and venous plasma ^{131}I radioactivities separately. Use of venous plasma samples can be observed to give a falsely high initial clearance value.

Arterial plasma, venous plasma and praecordial radioactivities following a 30 second intravenous infusion of $^{99\text{m}}\text{Tc}$ in a representative patient are shown in Figure 11. Although the calibrated praecordial count rate was consistently more representative of arterial than venous radionuclide concentration, it failed to predict accurately the arterial curve.

In 10 subjects studied with $^{99\text{m}}\text{Tc}$, arterial plasma concentration at 1 minute was 0.28 ± 0.08 fraction dose/litre (mean \pm 1 S.D.) and in 6 subjects studied with ^{131}I , arterial plasma concentration at 1

minute was 0.20 ± 0.06 fraction dose/litre (mean \pm 1 S.D.).

DISCUSSION

Thyroid follicular cells are probably exposed to a plasma concentration of ^{131}I and $^{99\text{m}}\text{Tc}$ in perifollicular capillaries which approximates to that in arterial plasma. It has been assumed by previous authors (Schultz and Zieve, 1957; Rall et al, 1964; Koutras and Sfontouris, 1966; Yamamoto et al, 1969) that homogenous mixing of an intravenous injection of radionuclide occurs within 2 minutes of administration allowing use of venous plasma samples after 2 minutes to approximate the arterial plasma perfusing thyroid. The author has shown firstly that this assumption is invalid and secondly that the systematic error introduced by using early venous plasma samples could result in the phenomenon of 'falling thyroidal ^{131}I clearance'. Although this data gives little indication of the circulatory physiology applicable to production of arterio-venous difference, both slow mixing of arterial and venous plasma in the brachial system and diffusion of plasma radionuclide into extravascular fluid of the arm are likely to be relevant factors.

Precise thyroid clearance studies in the early phase require correction for this error if venous plasma sampling is used. It was disappointing that praecordial (ventricular) counting failed to provide a suitable alternative to arterial cannulation. This probably resulted from variation in the extracardiac contribution to total praecordial counts which would occur with time and from patient to patient.

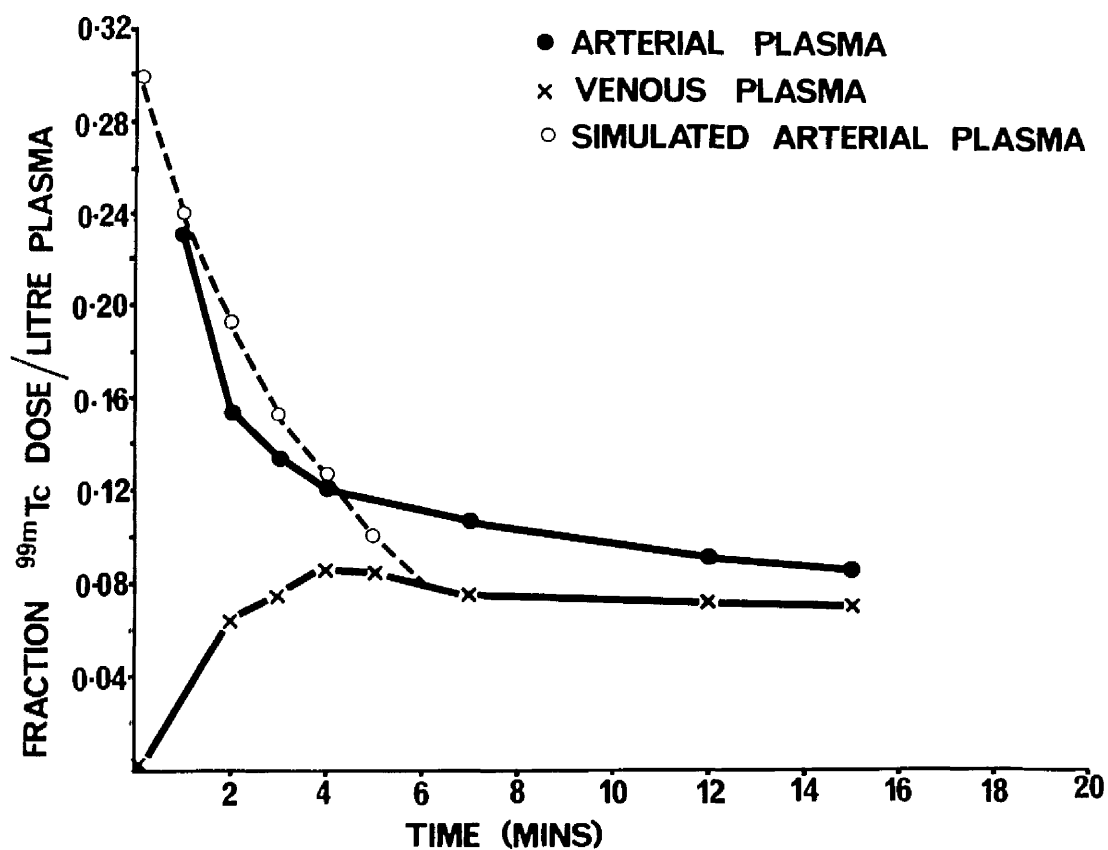


Figure 12: Arterial, venous and simulated arterial plasma radio-activities following an intravenous injection of ^{99m}Tc illustrating partial correction of the venous curve for arterio-venous difference.

It is interesting that the plasma concentration of ^{99m}Tc is higher than that of ^{131}I and this is probably a reflection of the plasma protein binding of ^{99m}Tc (Oldendorf et al, 1970; Hayes and Green, 1973).

To obtain maximum precision in thyroid clearance studies performed in the early phase with ^{131}I and ^{99m}Tc and reported in this thesis, an approximation of arterial radionuclide concentration was used during the first 6 minutes after injection. To the venous curve of each patient, an exponential curve was added with a starting value of 0.3 fraction dose/litre for ^{99m}Tc and 0.2 fraction dose/litre for ^{131}I which intersected the respective venous curves at 6 minutes (Figure 12). The systematic error of arterio-venous difference in each patient studied was therefore minimised though not entirely eliminated.

CHAPTER 6 - SUMMARY

A significant arterio-venous difference in plasma ^{131}I and $^{99\text{m}}\text{Tc}$ concentration has been noted at early intervals following their intravenous injection. It is suggested that the phenomenon of falling thyroid clearance of ^{131}I , found when venous samples are used for clearance calculation, results from this systematic error. Praecordial counting was found to be unsuitable for predicting arterial concentration of radionuclide.

SECTION 3

ASSESSMENT OF THYROIDAL IODIDE TRANSPORT
WITH TECHNETIUM

- | | |
|------------|--|
| Chapter 7 | Autoradiography of technetium-99 in rat
thyroid |
| Chapter 8 | Determination of the rates of accumulation
and loss of technetium-99m and iodide-131
from human thyroid glands in vivo |
| Chapter 9 | Thyroid uptake of technetium-99m |
| Chapter 10 | Effect of radioactive iodine therapy on the
activity of iodide transport as measured by
thyroid uptake of technetium-99m |

CHAPTER 7

AUTORADIOGRAPHY OF TECHNETIUM-99

IN RAT THYROID

This chapter describes studies to determine the distribution of technetium as pertechnetate in rat thyroid gland.

INTRODUCTION

The uptake of technetium as pertechnetate by the thyroid (Chapter 9) is currently accepted as a useful clinical index of thyroid function (Alexander et al, 1969) and an acceptable alternative to early thyroid uptake measurements using radioactive iodine (Shimmins et al, 1971). Although Pitt-Rivers and Trotter (1953) have shown that radioactive iodide is concentrated in follicular colloid, the intrathyroidal site of pertechnetate concentration has not been previously determined.

This chapter reports results of autoradiographic studies with pertechnetate-⁹⁹Tc in rat thyroid performed for the author by Mr. M. Small, technician in the Department of Oral Pathology, Glasgow Dental Hospital.

Autoradiography The principal of tissue autoradiography is simple. Thin sections of tissue containing the radionuclide under study are placed in contact with a film of photographic emulsion. Ionisation in the emulsion induced by radiation results in the formation of silver grains in the distribution of radioactivity. Both tissue section and emulsion may then be processed so that the relation of radioactivity (silver grains) to follicle and cell structure is

maintained. The main technical problem of autoradiography with pertechnetate relates to the lack of protein binding of radionuclide in thyroid since conventional processing of histological sections will leach out soluble pertechnetate ions. In this study therefore, a freeze-drying technique was used which prevented movement and loss of pertechnetate until exposure to the autoradiographic emulsion was completed.

MATERIALS AND METHODS

Five Sprague-Dawley albino adult male rats were used. Two rats received aminotriazole (0.1%) in drinking water for three weeks before the study; two other rats were fed a normal diet. Pertechnetate-⁹⁹Tc (300 uCi) was administered intraperitoneally to each animal and after one hour, one animal in each group received 10 mg sodium perchlorate by the same route. Ninety minutes after radionuclide administration and under ether anaesthesia, the thyroid and trachea were rapidly removed together, frozen in isopentane, cooled in liquid nitrogen to about -170°C, and sectioned (8 µm) on a cryostat in the darkroom without thawing. The frozen sections were placed directly on to slides previously covered with photographic emulsion (Rogers and Brown-Grant, 1971) and were exposed at -30°C for five days in a light-tight box. The emulsion was developed in D.19B (Kodak Ltd.), and fixed. Finally the preparations were stained by haematoxylin and eosin for examination under light microscopy. The procedure was repeated in one other rat given aminotriazole, though the sections were allowed to thaw before preparing autoradiographs.

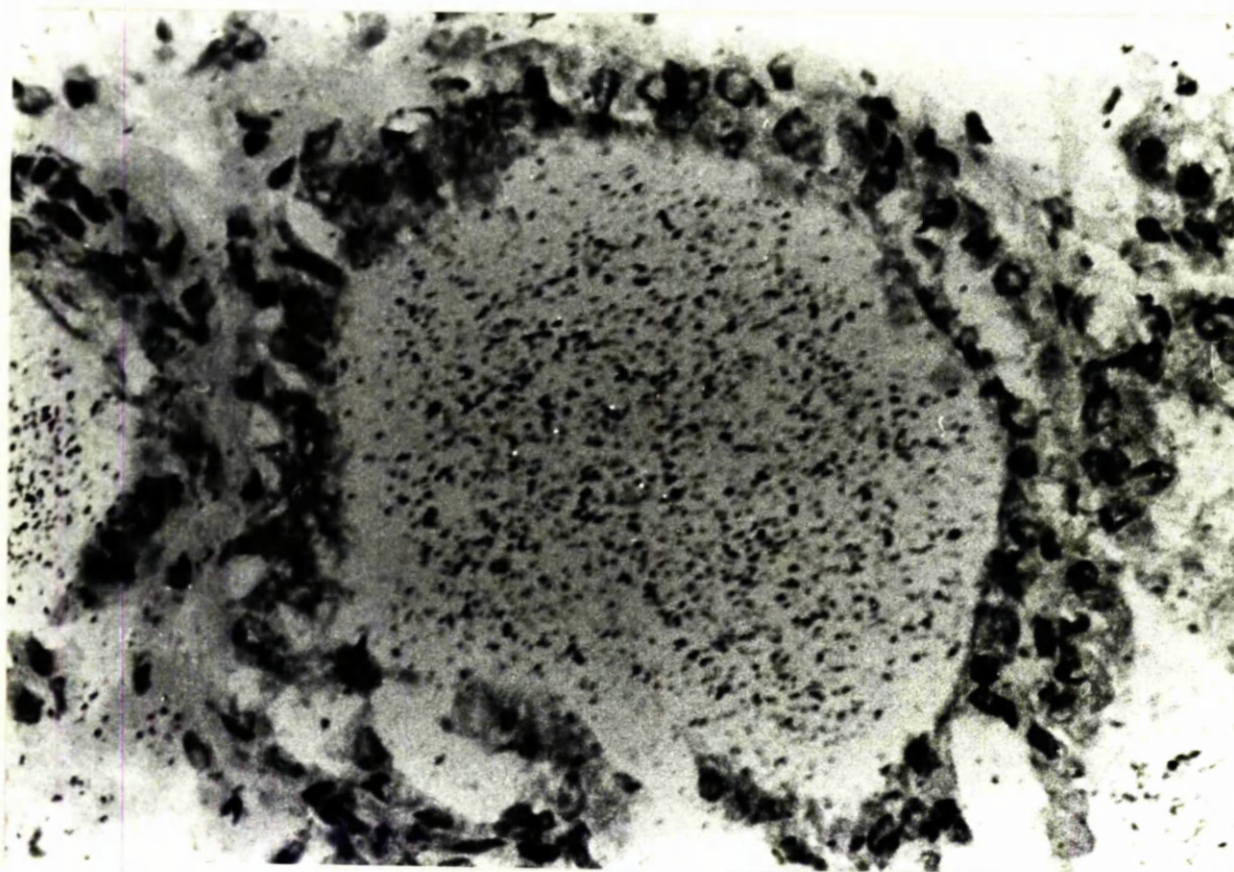


Figure 13: Autoradiographic reaction of ^{99}Tc primarily over the colloid of a normal rat thyroid follicle (x 500).

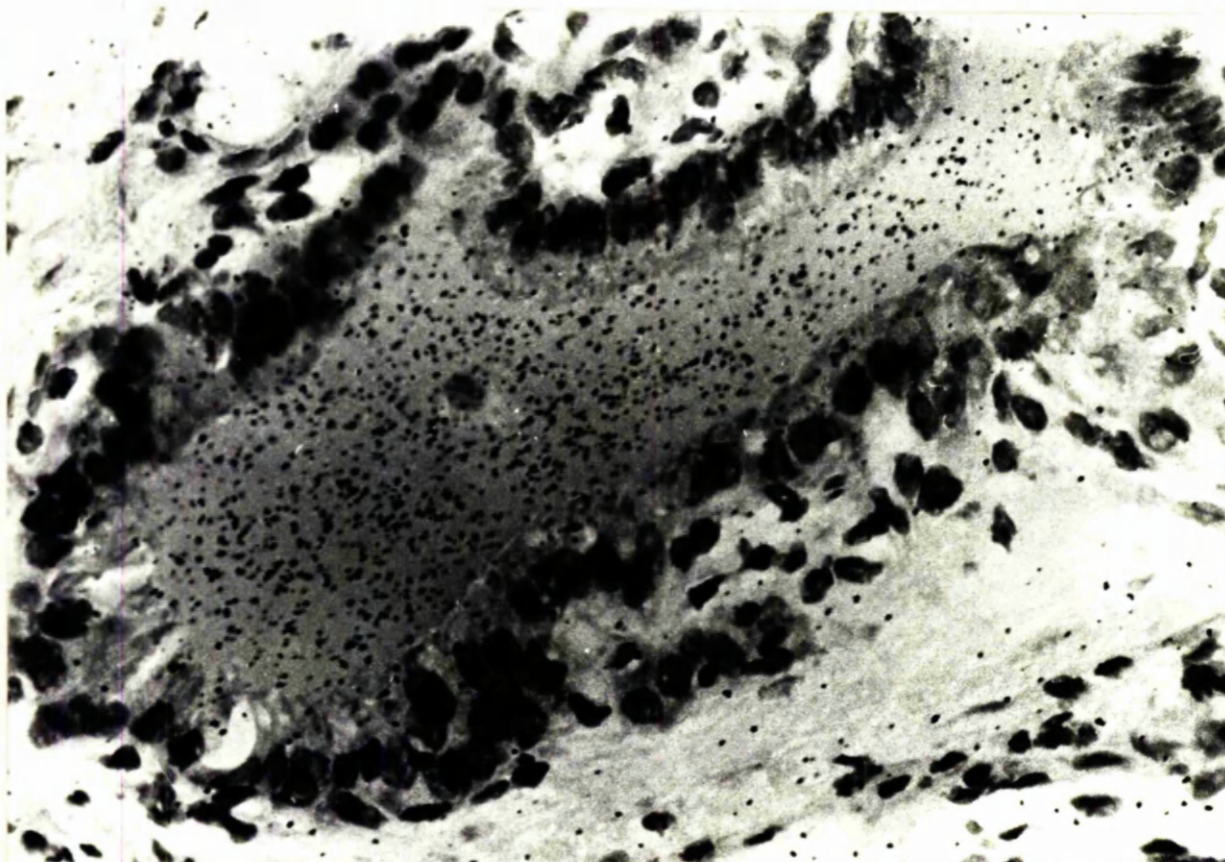


Figure 14: Rat thyroid stimulated by blocking iodide organification. Autoradiographic reaction of ^{99}Tc over the colloid with little reaction over enlarged follicular cells.

RESULTS

The follicular arrangement of the thyroid can be recognized on histological examination of both unstimulated (Figure 13) and stimulated glands (Figure 14). Autoradiographs from those animals not given perchlorate disclosed that the major site of pertechnetate concentration was the colloid. There was little radioactivity over follicular cells or interstitium. In animals given perchlorate, no significant radioactivity was seen in colloid confirming discharge of radionuclide from the thyroid. Sections of gland which were allowed to thaw before preparing autoradiographs failed to show localisation of pertechnetate as described above. These sections showed a diffuse image over the entire gland.

DISCUSSION

The presence of an autoradiographic image over follicular colloid in rat thyroid indicates the selective concentration of pertechnetate within this structure. Pertechnetate discharge from colloid by perchlorate, first reported by Harper et al (1962) has been confirmed. This resemblance, both to the localisation (Pitt-Rivers and Trotter, 1953; Doniach and Logothetopoulos, 1955a) and perchlorate discharge (Stanbury and Wyngaarden, 1952) of thyroidal radioactive iodide, confirms a basic similarity in transport of pertechnetate and iodide by the thyroid, first demonstrated by Schindler et al (1966) in thyroid kinetic studies using both radionuclides. The author considers these findings justify his subsequent extension of thyroid model theory from iodide (Wollman and Reed, 1962a) to technetium as pertechnetate.

CHAPTER 8

DETERMINATION OF THE RATES OF ACCUMULATION AND LOSS OF TECHNETIUM-^{99m} AND IODIDE-¹³¹ FROM HUMAN THYROID GLANDS IN VIVO

This chapter describes a study of pertechnetate-^{99m}Tc and iodide-¹³¹I kinetics in thyroid over a 20 minute period following intravenous radionuclide injection. The data has been analysed graphically, digitally and with an analogue computer.

INTRODUCTION

Thyroid metabolism of iodide can be conveniently described in terms of a hypothetical two-compartment model if iodide organification is blocked and a three-compartment model if iodide organification is proceeding (Chapter 4). Pertechnetate-⁹⁹Tc accumulation in the colloid of rat thyroid (Chapter 7) confirms an anatomical similarity in compartmental handling of iodide and pertechnetate by thyroid and justifies the extension of model theory to thyroid kinetics of pertechnetate. Since this radionuclide is virtually unorganified in thyroid, a two-compartment model is operative and its successful application to data obtained in vivo on the uptake of pertechnetate-^{99m}Tc by human thyroid has been reported by Shimmins et al (1968b).

To obtain further insight into handling of pertechnetate-^{99m}Tc (^{99m}Tc) by thyroid in normal and diseased patients, the author has recorded both thyroidal uptake and venous plasma concentration of ^{99m}Tc over 20 minutes following its intravenous injection in a series

of normal and thyrotoxic patients. From this data, the two kinetic parameters which describe the model have been calculated for each subject; thyroid unidirectional clearance (C.L/min), corresponding to the initial flux of ^{99m}Tc ions into thyroid from plasma, and exit rate-constant (K_{TP} , min^{-1}), corresponding to the continuous efflux of ^{99m}Tc from thyroid to plasma. Kinetic parameters of radioactive iodide-131 (^{131}I) handling by thyroid were measured simultaneously in each subject; unidirectional clearance (C) and exit rate-constant (K_{TP}) for ^{131}I when iodide organification was blocked with carbimazole and net clearance of ^{131}I when iodide organification was normal. This net clearance represents the balance between ^{131}I ions entering and leaving thyroid.

Data analysis has been performed in three ways. .

1. By the graphical technique of Shimmins et al (1968b).
2. By a modification of the above using a digital computer to perform a least-squares fit of the data.
3. By analogue computer.

Consisting of an electrical network which can simulate a biological model, the analogue computer is subject to minor intrinsic errors and should theoretically permit more accurate evaluation of the rate-constants of a model than graphical methods.

The complete data permits a unique comparison between kinetics of ^{99m}Tc and ^{131}I in thyroid.

MATERIALS AND METHOD

Carrier-free sodium pertechnetate- ^{99m}Tc (^{99m}Tc) and carrier-free

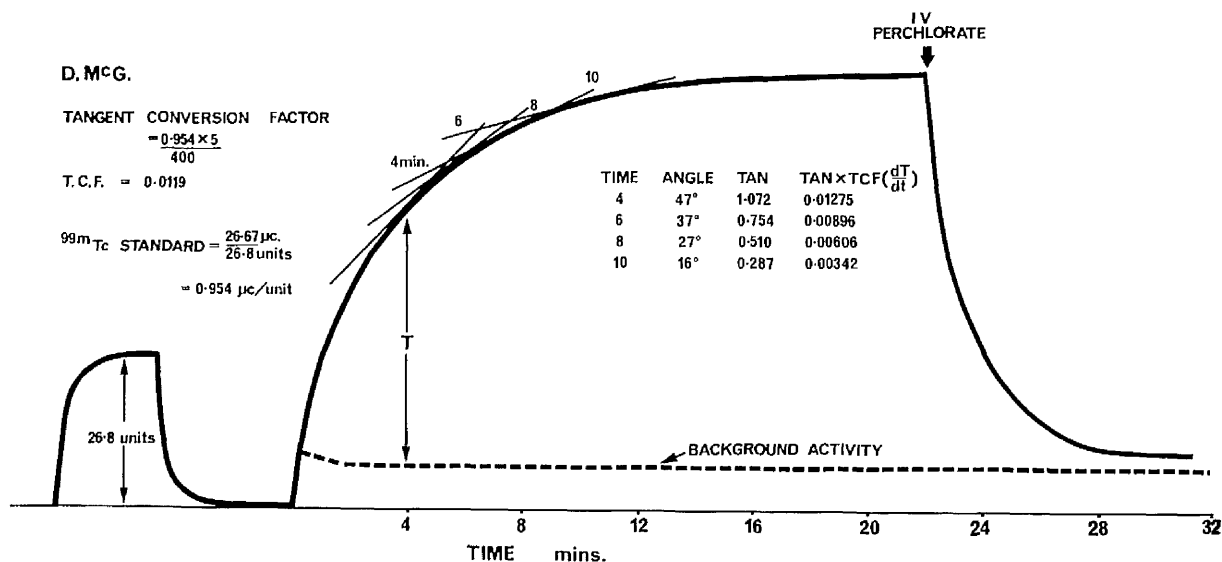


Figure 15: Diagrammatic analysis of ^{99m}Tc dose standard and thyroid uptake curve of ^{99m}Tc recorded automatically. In addition, discharge of thyroidal ^{99m}Tc is shown after IV perchlorate injection. The rate of increase of thyroidal ^{99m}Tc content, $\frac{dT}{dt}$, is obtained by correcting the measured slope of tangent to this thyroid uptake curve. The correction factor consists of a scaling correction as numerator and administered dose (e.g. 400 μCi) as denominator to give $\frac{dT}{dt}$ in fract. admin. dose units. The scaling correction is the number of μCi in one length of time scale (1 minute) to adjust for non-linear plot - in this case 0.954×5 .

sodium iodide- ^{131}I (^{131}I) were used (Radiochemical Centre, Amersham). Uptake of $^{99\text{m}}\text{Tc}$ and ^{131}I by thyroid and venous plasma concentration of each radionuclide were recorded in 32 patients; 7 normal volunteers with no evidence of thyroid disease, 13 patients with untreated thyrotoxicosis and 12 thyrotoxic patients currently taking 45 mg carbimazole daily.

Instrumentation

The modified directional counter (Chapter 5) was connected to isotope counting equipment feeding two scaling circuits, each connected via a ratemeter to a separate potentiometric recorder. One circuit counted ^{131}I optimally, the other counted $^{99\text{m}}\text{Tc}$. Special collimation reduced the contribution to total neck counts of extrathyroidal radioactivity (ETA) to approximately 2% of administered dose for both radionuclides (Chapter 5).

The system was calibrated using suitable standards of ^{131}I and $^{99\text{m}}\text{Tc}$ in a lucite thyroid phantom. Cross-activity between channels was negligible as administered ^{131}I radioactivity was only 1/6th of $^{99\text{m}}\text{Tc}$ radioactivity. Two ml plasma samples were counted in a well-type scintillation counter.

Procedure

Patients lay on a couch and the special collimator was carefully positioned over the thyroid. Following the simultaneous intravenous injection of 400 μCi $^{99\text{m}}\text{Tc}$ and 25 μCi ^{131}I , the curves of thyroidal plus extrathyroidal radioactivity for each radionuclide were continuously recorded for 20 minutes (Figure 15). Venous blood samples, taken at 3, 5, 7, 10, 15 and 20 minutes after injection,

allowed measurement of plasma concentrations of each radionuclide. Sodium perchlorate (50 mg) was injected intravenously at 20 minutes (for preparation - see Appendix 2) and recording of total neck radioactivity of ^{99m}Tc and ^{131}I was continued. Although perchlorate injection resulted in a rapid 'washout' of thyroidal ^{99m}Tc in each subject studied, 'washout' of thyroidal ^{131}I occurred only in those subjects currently on carbimazole. When ^{99m}Tc 'washout' was complete, a further 400 μCi ^{99m}Tc was injected intravenously. Measurement of the resulting increase in total neck radioactivity over background radioactivity during the subsequent 10 minutes allowed approximate quantitation of ^{99m}Tc ETA with time. When ^{131}I ETA was required for analysis, the corresponding ^{99m}Tc value was substituted since the difference between either ETA measurement at any one time is marginal (Chapter 5) using this collimation.

ANALYSIS OF KINETIC DATA

The model employed for ^{99m}Tc data analysis was a two-compartment model (Chapter 4). This model was also applied to ^{131}I data when iodide organification was blocked with carbimazole.

Analysis was performed in all subjects by graphical and digital means but in 10 thyrotoxic patients, analogue computation was used in addition.

Graphical analysis - ^{99m}Tc

In a two-compartment thyroid model relating to ^{99m}Tc .

$$T(t) = \text{Total } ^{99m}\text{Tc content of thyroid at time 't'}$$

(fract. admin. dose).

- $P(t)$ = ^{99m}Tc plasma concentration at 't'
(fract. dose/litre).
- C = Thyroid unidirectional clearance of ^{99m}Tc
(litres/min).
- K_{TP} = Fraction of thyroidal ^{99m}Tc diffusing to
plasma/min (min^{-1}).

The linear differential equation describing net transfer of ^{99m}Tc into thyroid is:

$$dT/dt = C \cdot P(t) - K_{TP} \cdot T(t) \text{ --- 1}$$

Divide this equation by $P(t)$.

$$\frac{dT/dt}{P(t)} = C - K_{TP} \cdot \frac{T(t)}{P(t)} \text{ --- 2}$$

Assuming C and K_{TP} are constant, equation 2 is the equation of a straight line with:

$$\frac{dT/dt}{P(t_i)} = \text{net thyroid clearance of } ^{99m}\text{Tc at time 't_i'.$$

$$\frac{T(t_i)}{P} = \text{thyroid/plasma ratio of } ^{99m}\text{Tc at time 't_i'.$$

The thyroid plasma ratio $\frac{T(t_i)}{P}$, measured when thyroid and plasma pools are in equilibrium, is termed the T_s ratio and is a special case of $\frac{T(t_i)}{P}$.

From the thyroid uptake curve of ^{99m}Tc in the representative thyrotoxic patient D.M.G. (Figure 15) and corresponding plasma concentration curve of ^{99m}Tc suitably corrected for arterio-venous difference (Chapter 5), we can calculate dT/dt for several times 't_i' after ^{99m}Tc injection (i.e. slope of the tangent to uptake curve), $T(t_i)$ the thyroid uptake, and $P(t_i)$ the corresponding plasma concentration

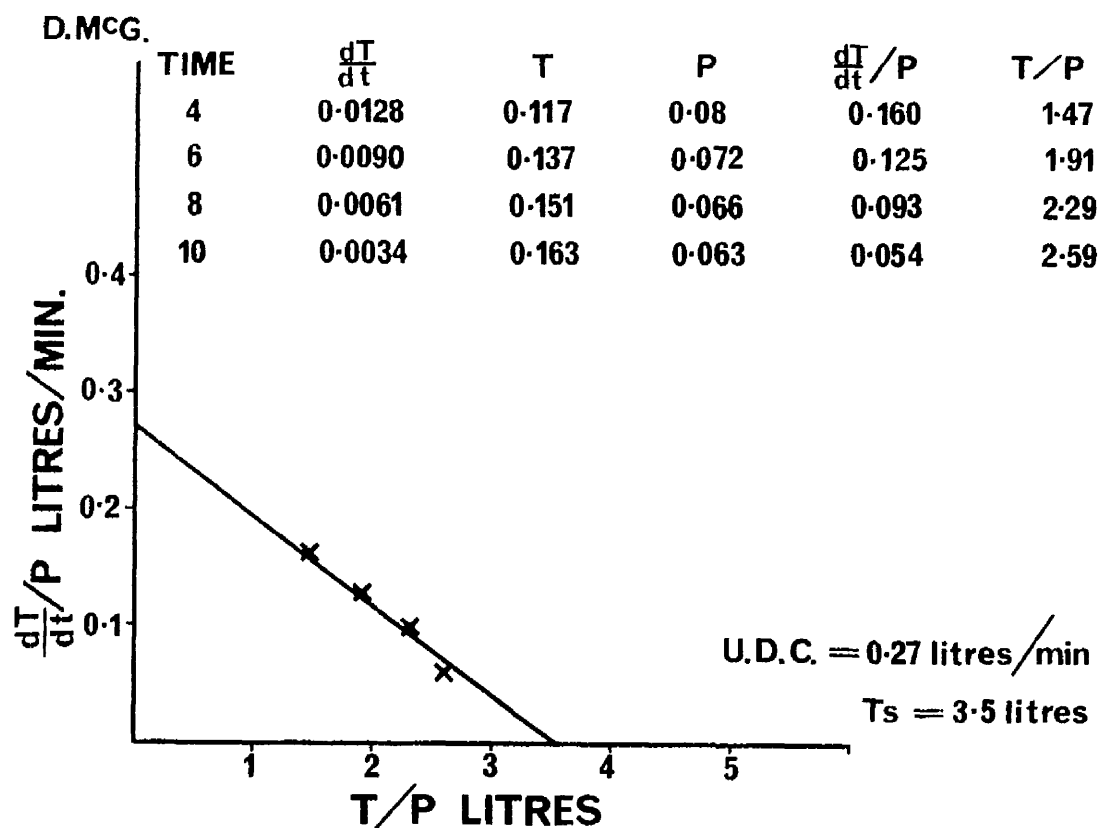


Figure 16: Straight-line analysis graph plotting $dT/dt/P$ against T/P for various times after IV ^{99m}Tc injection. The unidirectional clearance is represented by the net clearance $dT/dt/P$ at zero time and the T_s ratio by T/P at equilibrium i.e. when the thyroid net clearance of ^{99m}Tc is zero.

of ^{99m}Tc . Kinetic rate-constants were subsequently calculated from this data as follows.

1. Graphical

Using equation 2 and graphing $dT/dt/P(t_i)$ against $\frac{T(t_i)}{P}$ (Figure 16), we obtain a straight line whose intercepts are thyroid T_s ratio and unidirectional clearance (i.e. net clearance at zero time). Also from equation 2, since $dT/dt/P(t_i)$ is zero when thyroid and plasma ^{99m}Tc pools are in equilibrium, we obtain

$$K_{TP} = \frac{C}{T_s}$$

2. Digital

A Wang 700 digital calculator was employed to obtain the best line fit of data ($dT/dt/P$ against T/P) using a least-squares programme. In addition to precise calculation of slope (K_{TP}) and y-intercept of the line (C), an estimate of random error for each parameter was obtained.

Graphical Analysis - ^{131}I

When iodide organification was completely blocked with carbimazole, the above methods were used to calculate thyroid unidirectional clearance, T_s and K_{TP} for ^{131}I .

When iodide organification was proceeding normally, the net thyroid clearance of ^{131}I was measured by dividing the increase in thyroidal ^{131}I from 10 - 20 minutes after radionuclide injection by the average plasma ^{131}I concentration during this period. The first 10 minutes of the thyroid uptake curve of ^{131}I were ignored to minimise the systematic error of arterio-venous difference.

Graphical Analysis - Calculation of K'_{TP}

Following intravenous injection of sodium perchlorate, ^{99m}Tc discharged from thyroid at a mono-exponential rate (Figure 15). Thyroidal ^{131}I also discharged mono-exponentially when iodide organification was blocked. The exponential discharge rate K'_{TP} was calculated by plotting, on a log-linear scale for each radionuclide, the points of difference between observed thyroid radioactivities and asymptotes.

Analysis with Analogue Computer

Technology and circuit diagram used are fully described in Appendix 2. For each patient studied, the basic procedure was firstly to adjust computer plasma radioactivity to fit the observed radioactivity curve of ^{99m}Tc . Secondly, the computer thyroid curve was superimposed on the observed thyroid curve of ^{99m}Tc uptake by suitable adjustment of computer constants C and K_{TP} . When a 'best-fit' of thyroid curves was obtained, the absolute values of C and K_{TP} were obtained from the computer.

ERRORS

Random Errors

Measurement of ^{131}I or ^{99m}Tc kinetic parameters by graphic analysis is subject to many random errors. These include statistical variation in ratemeter output, errors in counting plasma radioactivity, variable neck-collimator distances, inaccurate dose administration and visual estimation of both slope of tangent to thyroid uptake curve and straight line in graphic analysis. Assuming that behaviour of ^{99m}Tc in thyroid

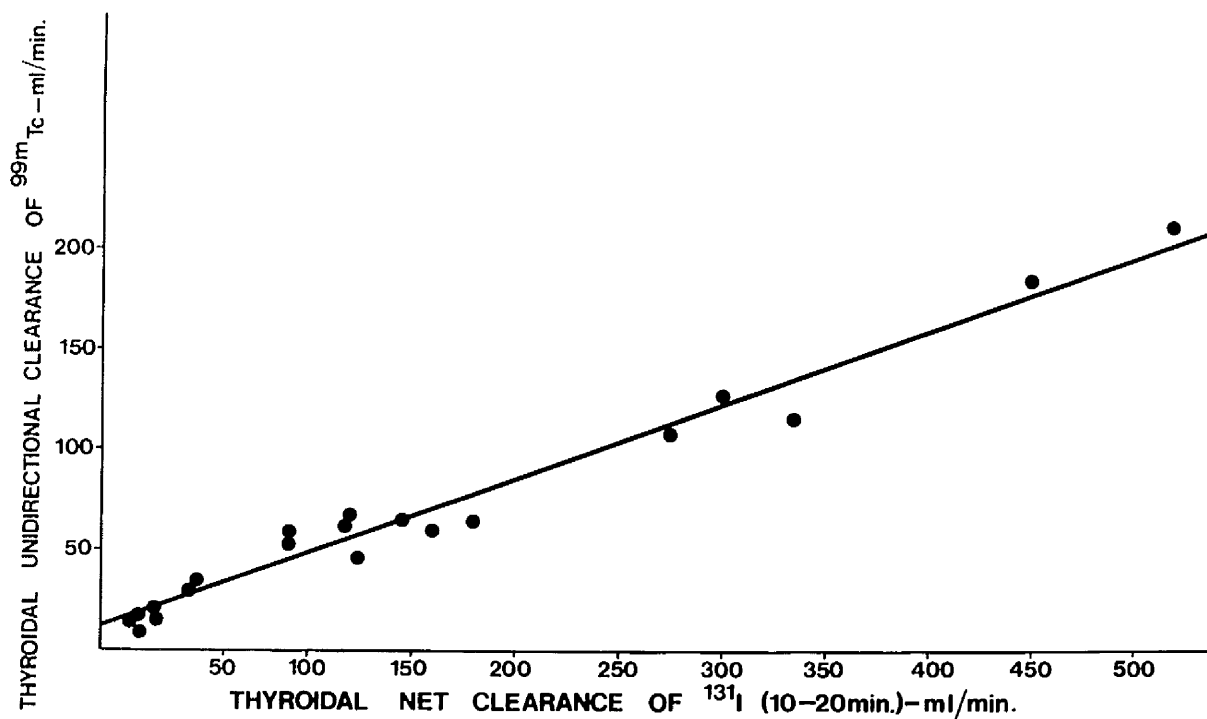


Figure 17: The correlation between thyroidal unidirectional clearance of $^{99\text{m}}\text{Tc}$ and the 10 - 20 minute net clearance of ^{131}I measured simultaneously in 7 euthyroid and 13 thyrotoxic subjects. The regression equation was $y = 0.38x + 0.009$ and $r = 0.98$.

TABLE 5

SUMMARISED RESULTS OF THYROID KINETIC ANALYSIS USING ^{99m}Tc AND ^{131}I

IN SUBJECTS WITH NORMAL UTILISATION OF TRAPPED IODIDE

SUBJECTS	^{99m}Tc		^{131}I
	UNIDIRECTIONAL CLEARANCE (L/min)	EXIT-RATE CONSTANT (min ⁻¹)	NET CLEARANCE (L/min)
Euthyroid (n = 7)	0.019 \pm 0.008	0.069 \pm 0.021	0.028 \pm 0.013
Thyrotoxic (n = 13)	0.093 \pm 0.053	0.081 \pm 0.027	0.222 \pm 0.138

Mean value and 70% confidence limits are shown

is in accordance with a two-compartment model, the error of C and K_{TP} calculation given by digital calculation indicates total random error (TRE). The TRE (70% confidence limits) for C was $13 \pm 5.0\%$ (mean \pm LSD) for ^{99m}Tc in 6 euthyroid and 13 thyrotoxic patients and $10 \pm 4.0\%$ (mean \pm LSD) for ^{131}I in 9 thyrotoxic patients. The TRE (70% confidence limits) for K_{TP} in the same patients was $18 \pm 6\%$ (mean \pm LSD) for ^{99m}Tc and $18 \pm 8\%$ (mean \pm LSD) for ^{131}I .

Systematic Errors

The most important systematic error is arterio-venous difference. Appropriate correction reduces this error from 15% to 5% approximately (Chapter 6).

RESULTS

There was an excellent correlation ($r = 0.99$) between results of thyroid kinetic analysis (C and K_{TP}) calculated by graphical and graphical/digital methods. The digital results alone are documented in this results section since they are mathematically precise and include random error quantitation.

A correlation between thyroid unidirectional clearance (UDC) of ^{99m}Tc and net clearance of ^{131}I is shown in Figure 17. The regression equation ($y = 0.38x + 0.009$) showed ^{99m}Tc UDC to be approximately 1/3rd the value of ^{131}I net clearance with $r = 0.98$. The gradient mean \pm LSD was 0.38 ± 0.016 . Results are summarised according to thyroid status in Table 5.

A correlation between UDC of both ^{99m}Tc and ^{131}I measured in 14 thyrotoxic patients currently on carbimazole therapy is not illustrated graphically (results in Table A, Appendix 1). The regression equation

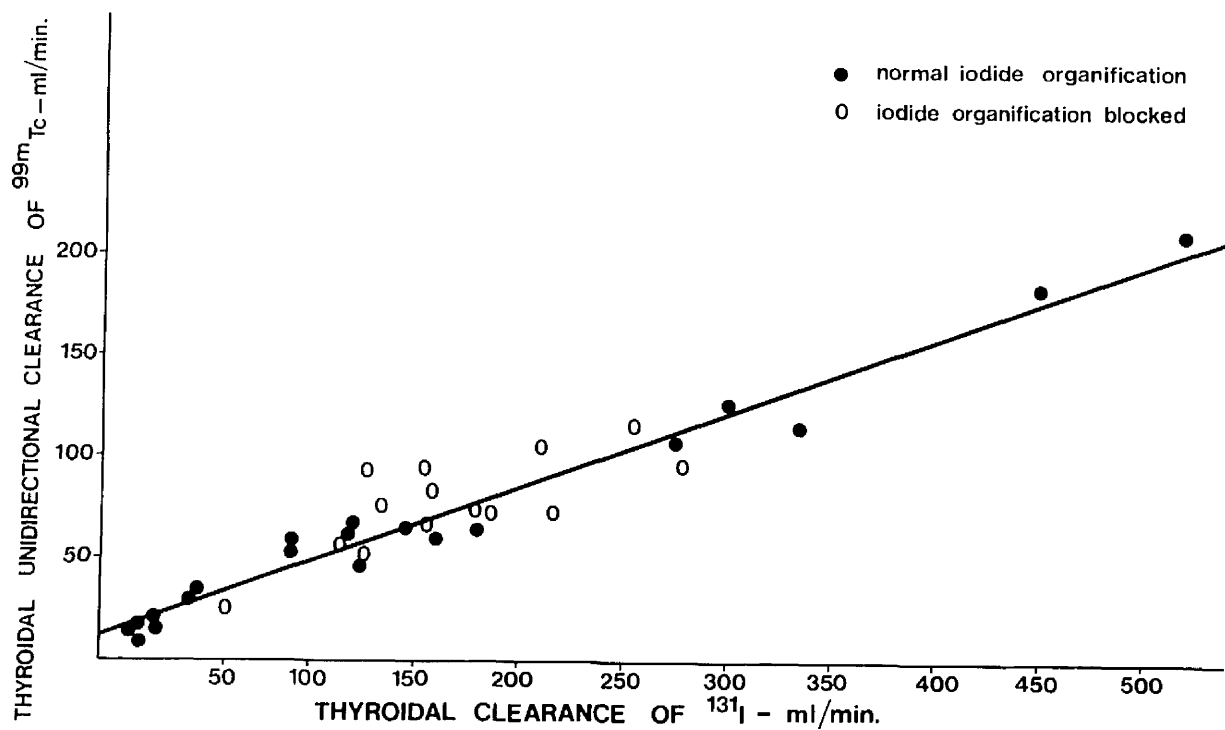


Figure 18: In this figure, simultaneous measurements of thyroidal unidirectional clearance (UDC) of both ^{99m}Tc and ^{131}I in 14 thyrotoxic patients on carbimazole (open circles) are superimposed on the correlation between thyroidal UDC of ^{99m}Tc and 10 - 20 minute net clearance of ^{131}I shown in Figure 17 (closed circles). The combined regression equation is $y = 0.37x + 0.011$ and $r = 0.95$.

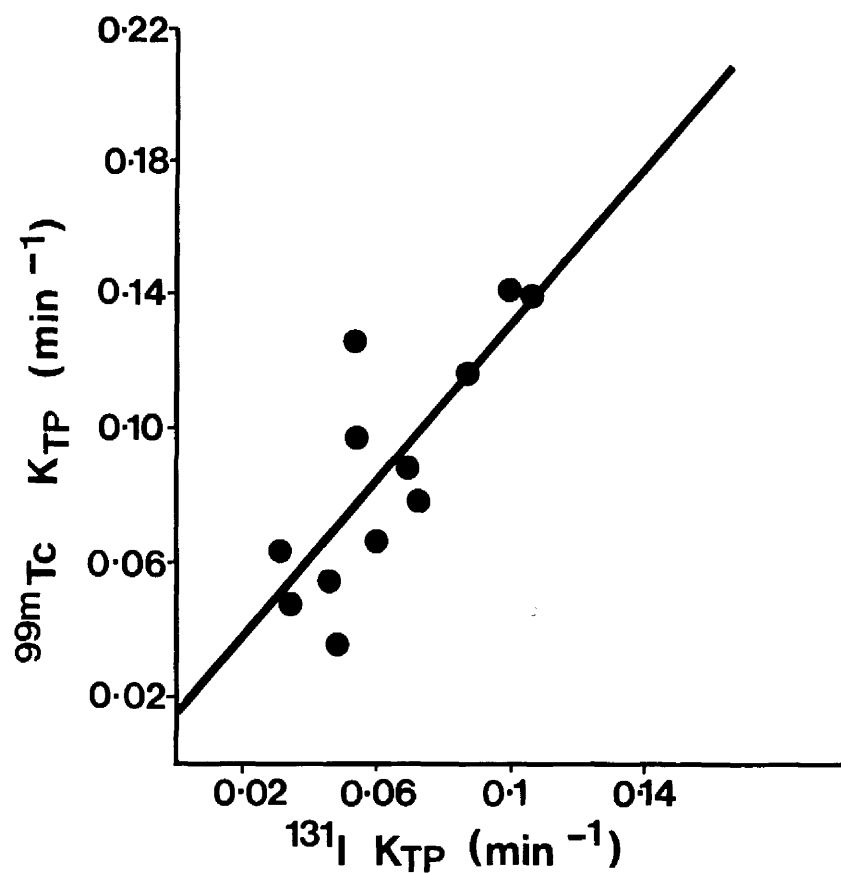


Figure 19: Correlation between the thyroid exit-rate constants (K_{TP}) of ^{99m}Tc and ^{131}I measured in subjects currently on carbimazole. The regression equation was $y = 1.17x + 0.014$ with $r = 0.79$.

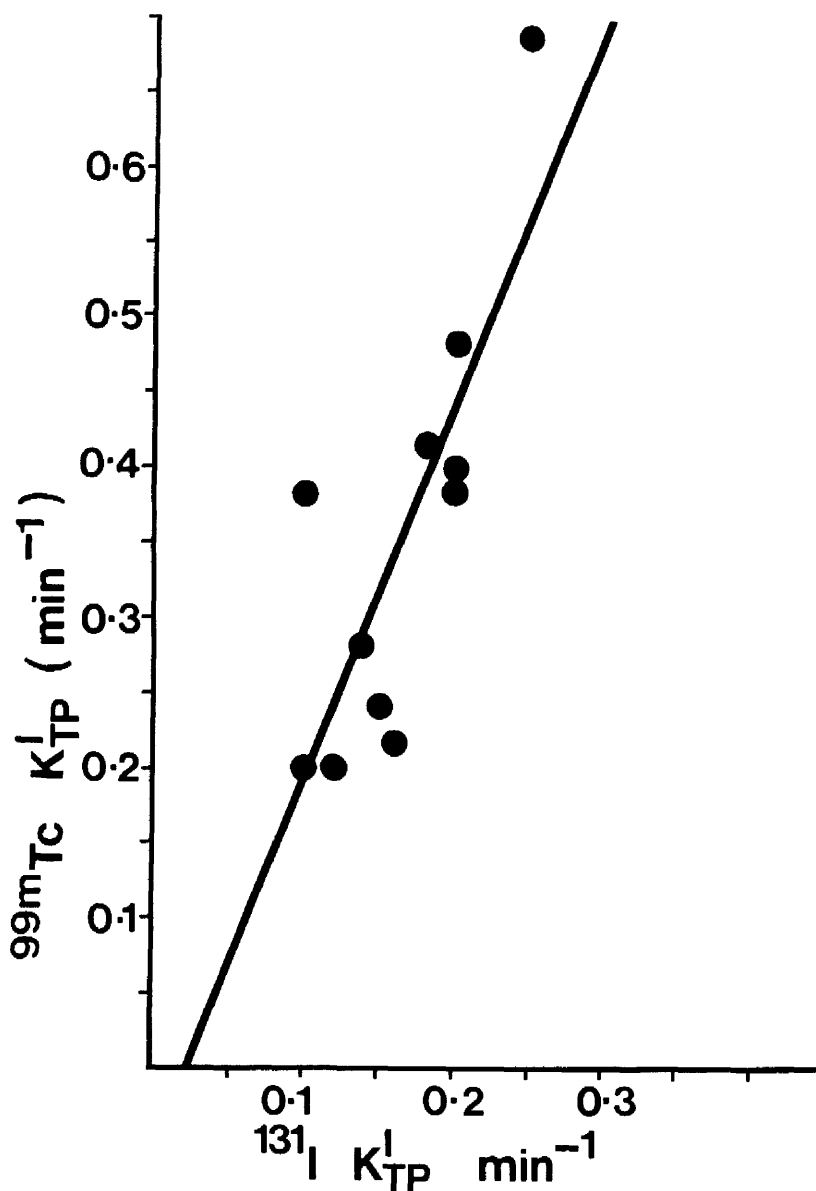


Figure 20: Correlation between the perchlorate-induced exit-rate constants (K_{TP}^I) of thyroidal ^{99m}Tc and ^{131}I measured in subjects currently on carbimazole. The regression equation was $y = 2.56x - 0.01$ and $r = 0.81$.

($y = 0.31x + 0.02$) again indicated that the ^{99m}Tc parameter was approximately 1/3rd the value for ^{131}I ; r was 0.74 and the gradient mean \pm LSD was 0.31 ± 0.082 . Since the gradient mean for the group who were not on carbimazole (0.38) is within LSD of the gradient mean for those on carbimazole (0.31 ± 0.082), it is unlikely that carbimazole administration altered the relationship between ^{99m}Tc and ^{131}I clearances. In Figure 18, the results obtained from these thyrotoxic patients on carbimazole are superimposed upon Figure 17. The regression equation of combined data was $y = 0.37x + 0.011$ (where $y = ^{99m}\text{Tc}$ UDC and $x = ^{131}\text{I}$ net or UDC) with $r = 0.95$.

In Figure 19, a good correlation is shown between thyroidal K_{TP} of ^{99m}Tc and ^{131}I in 12 thyrotoxic patients. The regression equation was $y = 1.17x + 0.014$ ($y = ^{99m}\text{Tc}$ and $x = ^{131}\text{I } K_{\text{TP}}$) with $r = 0.79$. In Figure 20, measurements of K'_{TP} of ^{99m}Tc and ^{131}I in the same patients are also shown to correlate well, with a regression equation of $y = 2.56x - 0.01$ ($y = ^{99m}\text{Tc}$ and $x = ^{131}\text{I } K'_{\text{TP}}$) and $r = 0.81$. Surprisingly, correlation between $^{99m}\text{Tc } K_{\text{TP}}$ and K'_{TP} and $^{131}\text{I } K_{\text{TP}}$ and K'_{TP} were poor (Tables B and C respectively - Appendix 1).

Correlation was disappointing between equivalent values of kinetic indices obtained using the analogue computer with those obtained from graphical/digital analysis; $r = 0.86$ for C and 0.80 for K_{TP} measurement (Appendix 1, Tables D and E).

DISCUSSION

Measurement of the unidirectional clearance (UDC) of ^{99m}Tc by the thyroid in euthyroid and thyrotoxic patients demonstrates that this index of ^{99m}Tc influx into thyroid correlates well with, and

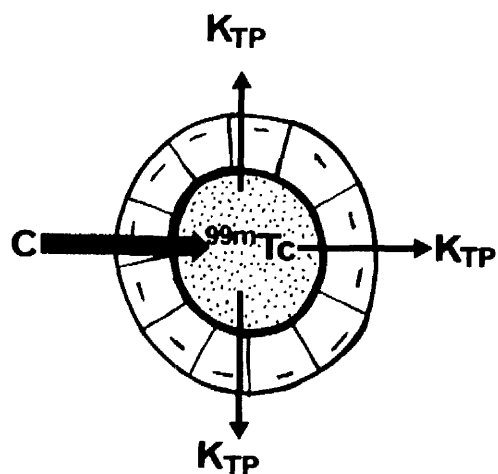
is directly proportional to, both thyroid net clearance of ^{131}I when iodide organification is normal and UDC of ^{131}I when iodide organification is completely blocked. This data has three important implications. Firstly, the results are consistent with an identical thyroid transport mechanism for each anion as described by Wolff (1963). Secondly, thyroid UDC of $^{99\text{m}}\text{Tc}$ appears to be a precise and direct index of the iodide transport activity of thyroid and will therefore provide the same quantitative information afforded by measurement of either thyroid net clearance (Rall et al, 1964) when iodide organification is normal or UDC of ^{131}I (Berson and Yalow, 1955). This accuracy is confirmed by the good separation obtained between thyrotoxic and euthyroid individuals studied using a $^{99\text{m}}\text{Tc}$ UDC measurement. The test was valueless for diagnosis of hypothyroidism, however, since data cannot be analysed if UDC is $< .01$ L/min. Clearly, measurement of thyroid UDC of $^{99\text{m}}\text{Tc}$ would appear most appropriate for assessment of iodide transport when the efficiency of iodide organification is in doubt. Thirdly, since carbimazole therapy has no effect on $^{99\text{m}}\text{Tc}$ kinetics (Schindler et al, 1966) the similar proportionality factor between $^{99\text{m}}\text{Tc}$ UDC and both ^{131}I UDC and net clearance measurements, argues that ^{131}I UDC and net clearance rates are similar when iodide organification is normal. Thus, the initial flux of ^{131}I ions into thyroid would appear equivalent to the resultant of influx and efflux at later times. This result, possible only if there is little unorganified ^{131}I in normal thyroid, implies an iodide organification rate near unity in the patients studied and confirms pioneer work of Berson and Yalow (1955).

The author's data conflicts directly with a current theory on thyroidal iodide metabolism in which an unorganified thyroidal iodide pool is postulated (Robertson et al, 1971; Shimmins, 1970; Alexander et al, 1971). This hypothesis requires ^{131}I UDC to be substantially larger than net clearance. Since it has been previously shown (Chapter 6) that the initial high thyroid clearance of ^{131}I which is observed in practice results from a systematic error of arterio-venous difference, the available evidence argues against this hypothesis.

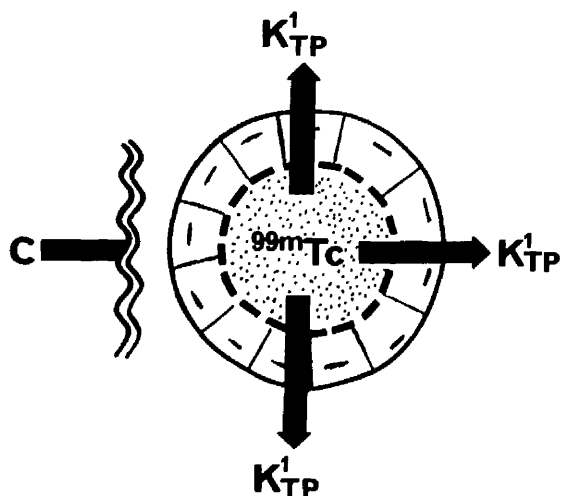
The observation that thyroid UDC of ^{131}I is approximately 2.5 x UDC of $^{99\text{m}}\text{Tc}$ in humans has not been previously reported and is surprising because Wolff (1964) has shown that K_m for $^{99\text{m}}\text{Tc}$ is lower than K_m for ^{131}I . The low rate of $^{99\text{m}}\text{Tc}$ transport into thyroid observed in vivo is conceivably a result of the binding of this radionuclide to plasma proteins (Hays and Green, 1973).

It is also of interest that Harden and Alexander (1967) found the salivary clearance of ^{131}I in humans to be 2.1 x the salivary clearance of $^{99\text{m}}\text{Tc}$. This finding in conjunction with the author's own data is compatible with a similar ionic transport mechanism in human thyroid and salivary glands and agrees with other studies (Wolff, 1964). Indeed it is likely that both $^{99\text{m}}\text{Tc}$ and ^{131}I share a common transport mechanism in these glands.

$^{99\text{m}}\text{Tc}$ and inorganic ^{131}I are distributed in the same anatomical compartment in thyroid (i.e. follicular colloid) and appear to be transported into thyroid by the same mechanism. The close correlation obtained between the exit-rate constants of $^{99\text{m}}\text{Tc}$ and ^{131}I , both before (K_{TP}) and after perchlorate injection (K'_{TP}), indicates a further



NORMAL ^{99m}Tc TRANSPORT



PERCHLORATE EFFECT

Figure 21: Diagrammatic representation of the effect of perchlorate on follicular function. Perchlorate firstly blocks the thyroid transport mechanism (C) for ^{99m}Tc and ^{131}I by competitive inhibition and secondly, exaggerates the 'leak' of unbound radio-nuclide from the follicle. This latter effect, demonstrated by an increase in K_{TP} to K'_{TP} , may result from a reduction in the follicular electro-chemical gradient induced by perchlorate.

basic similarity, namely in ionic diffusion, although ^{99m}Tc was clearly more active in this respect. In the analysis graph, the slope (K_{TP}) of plot $dT/dt/P$ against T/P was constant and independent of thyroid content of radionuclide (Figure 16). This confirms that K_{TP} is due to simple diffusion of radionuclide through thyroid follicular cells and probably results from the concentration gradient between follicle and interstitium. K'_{TP} after perchlorate is larger than K_{TP} by a factor of 2 to 3, however, and appears to represent a different phenomenon. The mono-exponential character of the 'washout' of both ^{99m}Tc and ^{131}I suggests a sudden 'release' of thyroid radionuclide followed by extremely rapid diffusion. Equivalent animal studies have both confirmed that perchlorate magnifies diffusion of ions from follicles (Scranton et al, 1969) and suggested (Wolff, 1968) that the effect results from depolarisation of thyroid cell membrane mediated by K^+ loss. The author's results could be in keeping with this theory and the hypothetical perchlorate effect has been depicted diagrammatically in Figure 21.

The analogue computer was found unsatisfactory for ^{99m}Tc rate constant measurement and a poor correlation ($r = .86$) with results from graphic analysis was obtained. In practice, precise curve fitting was difficult because more ^{99m}Tc was concentrated by thyroid in the first 2 minutes after injection than could be explained by the approximated arterial plasma curves. Although these latter curves were approximate, the author is convinced that their inherent error is small and considers it more likely that this phenomenon represents bolus trapping by thyroid of unbound plasma ^{99m}Tc in the immediate

post-injection phase. Further studies of this phenomenon are being pursued.

In conclusion, this in vivo study of the metabolism of ^{99m}Tc and ^{131}I in thyroid indicates clearly that thyroid UDC of ^{99m}Tc is an ideal substitute for, and is often preferable to, ^{131}I clearance parameters as an index of thyroidal iodide transport. Despite the undoubted clinical value of the ^{99m}Tc UDC measurement in the differential diagnosis of thyrotoxicosis, the elaborate protocol necessary, coupled with an insensitivity at low clearance rates, would seem to preclude its routine clinical use in investigation of thyroid function.

SUMMARY - CHAPTER 8

Using a simple directional counting technique, the thyroid transport function for ^{99m}Tc and ^{131}I was quantitated primarily using a combined graphical and digital analysis. Direct and equal proportionality found between thyroid unidirectional clearance of ^{99m}Tc and both thyroid net and unidirectional clearance of ^{131}I indicates firstly that unidirectional clearance of ^{99m}Tc is an index of thyroidal iodide transport and secondly, that little if any, recently transported iodide is unorganified in normal and thyrotoxic thyroid glands. A close correlation obtained between thyroidal exit-rate constants for ^{99m}Tc and ^{131}I both before (K_{TP}), and after perchlorate (K'_{TP}), indicates a similar ionic diffusion pathway from thyroid for each radionuclide. Perchlorate magnified this diffusion by a factor of 2 to 3.

CHAPTER 9

THYROID UPTAKE OF TECHNETIUM-99m

This chapter describes a method for measuring the thyroid uptake of pertechnetate- ^{99m}Tc using directional counting. Accuracy of the technique is assessed and possible roles for the measurement in clinical investigation are discussed.

INTRODUCTION

In Chapter 8, thyroid unidirectional clearance of pertechnetate- ^{99m}Tc (^{99m}Tc) was shown to be an accurate index of thyroidal iodide transport. This index was considered unsuitable for routine use in clinical investigation since its measurement was both time consuming and insensitive at low clearance rates. As an alternative index of thyroidal iodide transport, a simple method has been developed for measuring thyroid uptake of ^{99m}Tc using directional counting.

Thyroid uptake of ^{99m}Tc is an accepted clinical index of thyroid function but is usually measured using quantitative scintiscanning techniques (Shimmins et al, 1969a; Williams et al, 1971) or expensive gamma-scintillation cameras (Hurley et al, 1972). These specialised techniques are time consuming unless computer facilities are available (De Garreta et al, 1968).

Precise measurement of the ^{99m}Tc uptake of thyroid using directional counting requires correction for extrathyroidal neck radioactivity (ETA). This is of the order of 5 - 6% of administered radioactivity with the I.A.E.A. collimator (Shimmins et al, 1969a).

Previous workers found accurate measurement of ETA difficult with directional counting systems. Since the normal ^{99m}Tc uptake of thyroid is only 1 - 3% of dose, inaccurate uptake values were invariably obtained. Andros et al (1965) had limited success with a 'washout' technique using oral potassium perchlorate while Van't Hoff et al (1972) approximated ETA and found the test useful only when thyroid uptake was above normal.

The author's technique for measuring thyroid uptake of ^{99m}Tc eliminates the necessity for separate ETA measurement. Having reduced the contribution of ETA to total neck counts with special collimation (Chapter 5), the amount of ^{99m}Tc , dischargeable from thyroid, is measured after its 'washout' by intravenous sodium perchlorate injection. These adaptations reduced the range of uncertainty in measurement of this type of uptake of ^{99m}Tc by thyroid from 40% to 15%.

Results of studies on 119 patients are presented and possible roles for this test in thyroid investigation are discussed.

MATERIALS AND METHODS

Thyroid uptake of ^{99m}Tc , 15 minutes after injection, was measured in 99 patients; 27 normal volunteers with no evidence of thyroid disease, 28 patients with untreated thyrotoxicosis, 15 patients with primary myxoedema, 10 hypothyroid patients with Hashimoto's disease (the criterion for diagnosis was the presence of a strongly positive precipitin test for serum antithyroglobulin antibody), 16 patients with simple goitre and 3 patients with Pendred's syndrome.

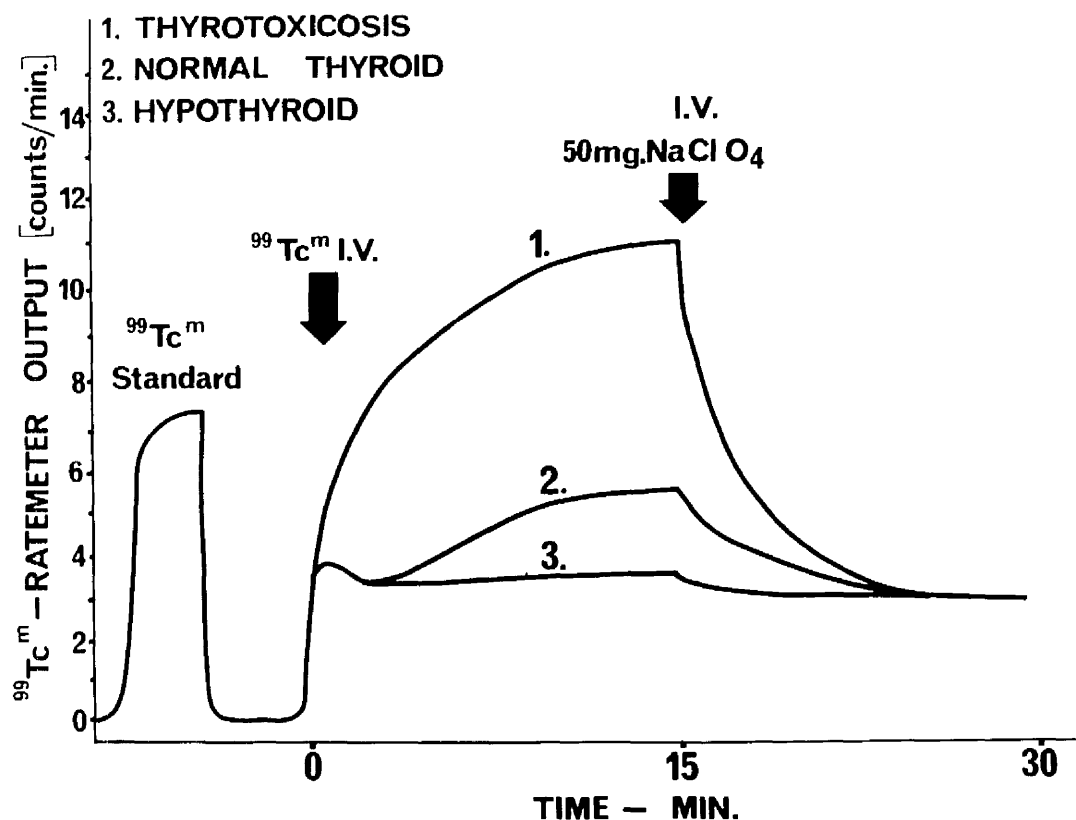


Figure 22: A trace record of neck radioactivity during measurement of the thyroid uptake of pertechnetate- ^{99m}Tc in a thyrotoxic (1), euthyroid (2), and hypothyroid (3) patient. 'Washout' of thyroidal ^{99m}Tc in each subject follows the IV injection of 50 mg of sodium perchlorate. The arrows indicate the times of injection of ^{99m}Tc and perchlorate; system calibration is shown with a dose standard in a thyroid phantom.

Instrumentation

The directional counting system, counting equipment and recorder have been previously described (Chapter 8). The system was calibrated using a standard of ^{99m}Tc in a thyroid phantom.

Procedure

Patients lay on a couch, the special collimator was carefully positioned over the thyroid and 400 - 800 μCi ^{99m}Tc was injected intravenously (Figure 22). Thyroidal plus extrathyroidal radioactivities were continuously recorded for 15 minutes. Sodium perchlorate (50 mg) was then injected intravenously (IV) and recording of total neck radioactivity continued until ^{99m}Tc 'washout' from thyroid was complete: this usually took 10 - 15 minutes. The difference between total radioactivity in neck before and after perchlorate discharge was considered to be thyroid uptake of ^{99m}Tc though it required a correction for fall in ETA during the period of discharge. This correction factor was found by measuring the fall in ETA between 15 and 30 minutes after an IV injection of ^{99m}Tc in 10 patients who had been given perchlorate orally, 1 hour before radionuclide. The correction was found to be 0.12% of administered dose ($\text{LSD} = \pm 0.05$) for a 10 minute discharge and 0.18% ($\text{LSD} = \pm 0.07$) for a 15 minute discharge.

In a further 4 normal and 16 thyrotoxic patients, recording of thyroid uptake and plasma radioactivity of ^{99m}Tc as described in the previous chapter was combined with a perchlorate discharge at 15 minutes, thereby allowing direct comparison between thyroid unidirectional clearance and uptake measurements.

Random errors include:

1. Statistical variation in output voltage of ratemeter

Found to be $< 1\%$.

2. Repositioning of patients

Allowing a reproducibility of collimator-neck distance of ± 0.5 cms, uptake was in error by 5%.

3. Injection calibration

Inaccuracy in estimating ^{99m}Tc dose resulted in the standard being in error by approximately 3%.

Systematic errors include:

4. ETA fall during perchlorate washout

Use of the correction factor described resulted in an error dependent on the absolute thyroid uptake of ^{99m}Tc . For an uptake of 0.5% dose, this error was 20%. For an uptake of 1% dose, it was 10% and for an uptake of 10% dose, the error was 1%.

5. Non-dischargeable thyroidal ^{99m}Tc

The absolute amount of ^{99m}Tc dose retained by thyroid after perchlorate in 11 thyrotoxic and 7 normal patients was approximately $0.5\% \pm 0.5$ (mean \pm LSD) and the error in measurement of thyroid uptake was $12.0\% \pm 10.0$ (mean \pm LSD).

Total random error in the ^{99m}Tc uptake and discharge phase were measured separately in each of 10 subjects and this error which includes technical inaccuracy and day to day variation in individuals was 10 - 15%.

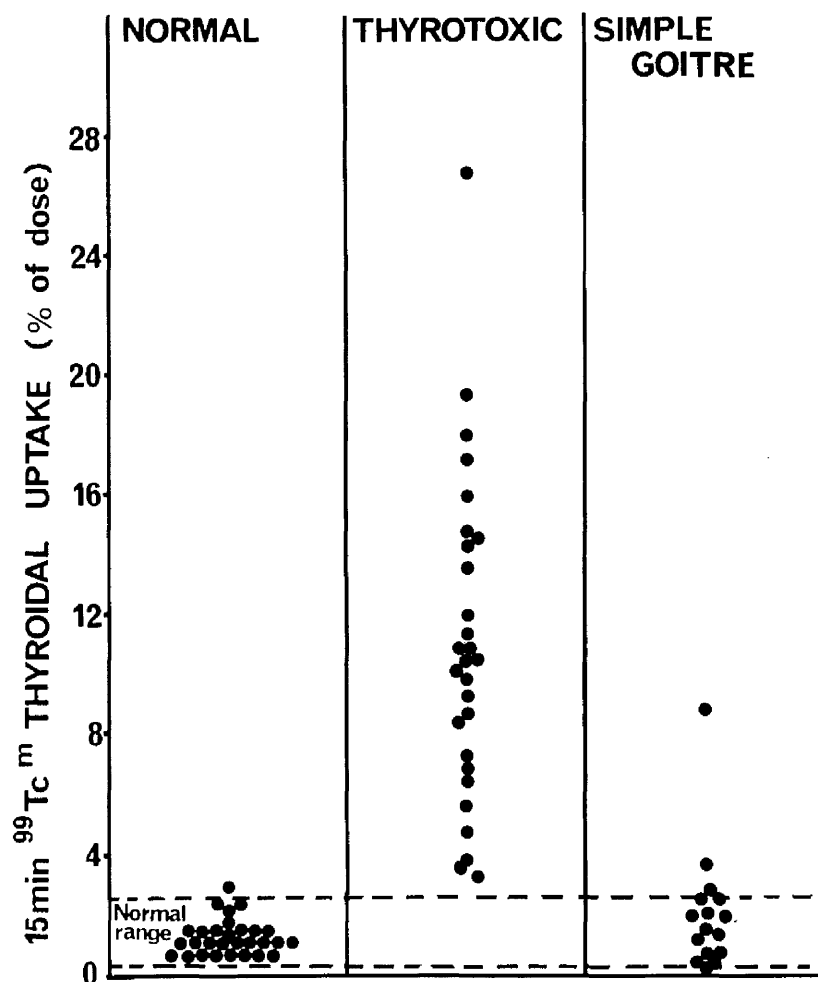


Figure 23: Values for the uptake of pertechnetate-^{99m}Tc by thyroid 15 minutes after IV injection in normal subjects (n = 27), patients with thyrotoxicosis (n = 28) and patients with simple goitre (n = 16). The horizontal broken lines indicate the normal range.

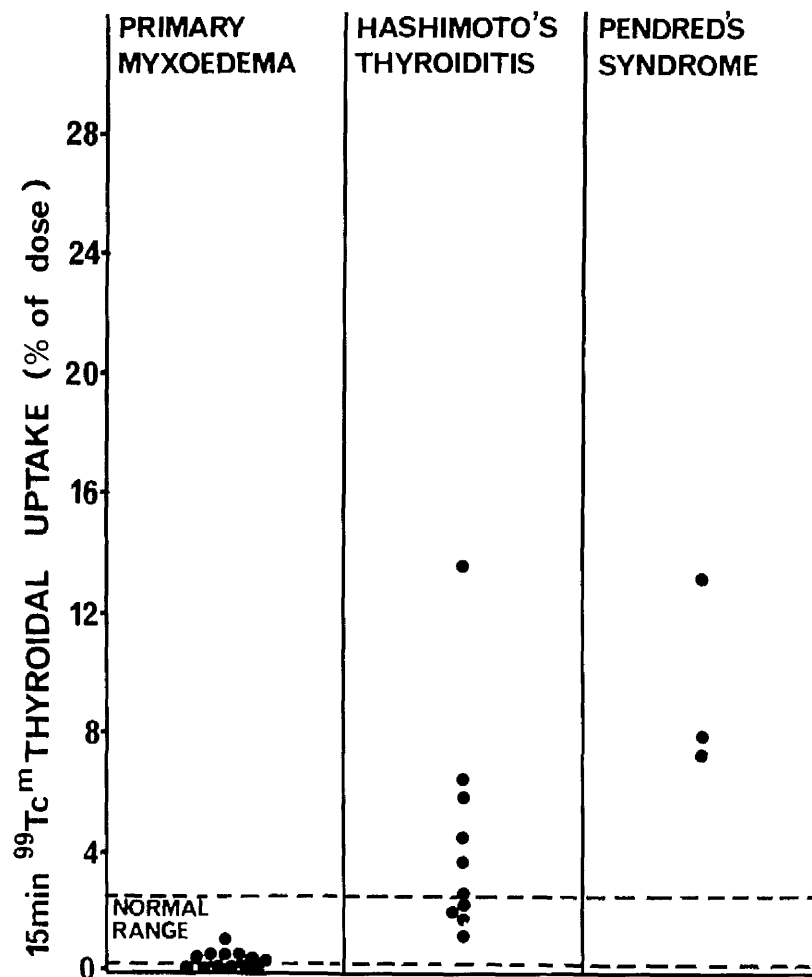


Figure 24: Values for the uptake of pertechnetate- $^{99\text{m}}\text{Tc}$ by thyroid, 15 minutes after IV injection in patients with primary myxoedema ($n = 15$), hypothyroid patients with Hashimoto's disease ($n = 10$) and patients with Pendred's syndrome ($n = 3$). The horizontal broken lines indicate the normal range.

TABLE 6

UPTAKE OF PERRHEINATE- ^{99m}Tc (^{99m}Tc) BY THYROID IN NORMAL

HUMAN SUBJECTS AND IN DISEASE STATES

SUBJECTS	THYROID UPTAKE OF ^{99m}Tc (% DOSE)		
	(MEAN \pm 1SD)	(RANGE)	
Normal (n = 27)	1.28 \pm 0.61	0.61 - 3.23	
Primary myxoedema (n = 15)	0.26 \pm 0.28	0.00 - 0.99	
Thyrototoxicosis (n = 28)	11.0 \pm 5.4	3.30 - 27.0	
Simple goitre (n = 16)	2.18 \pm 2.10	0.40 - 9.88	
Hashimoto's disease (n = 10)	4.50 \pm 3.70	1.50 - 13.70	
Pendred's syndrome (n = 3)	9.30	7.20 - 13.20	

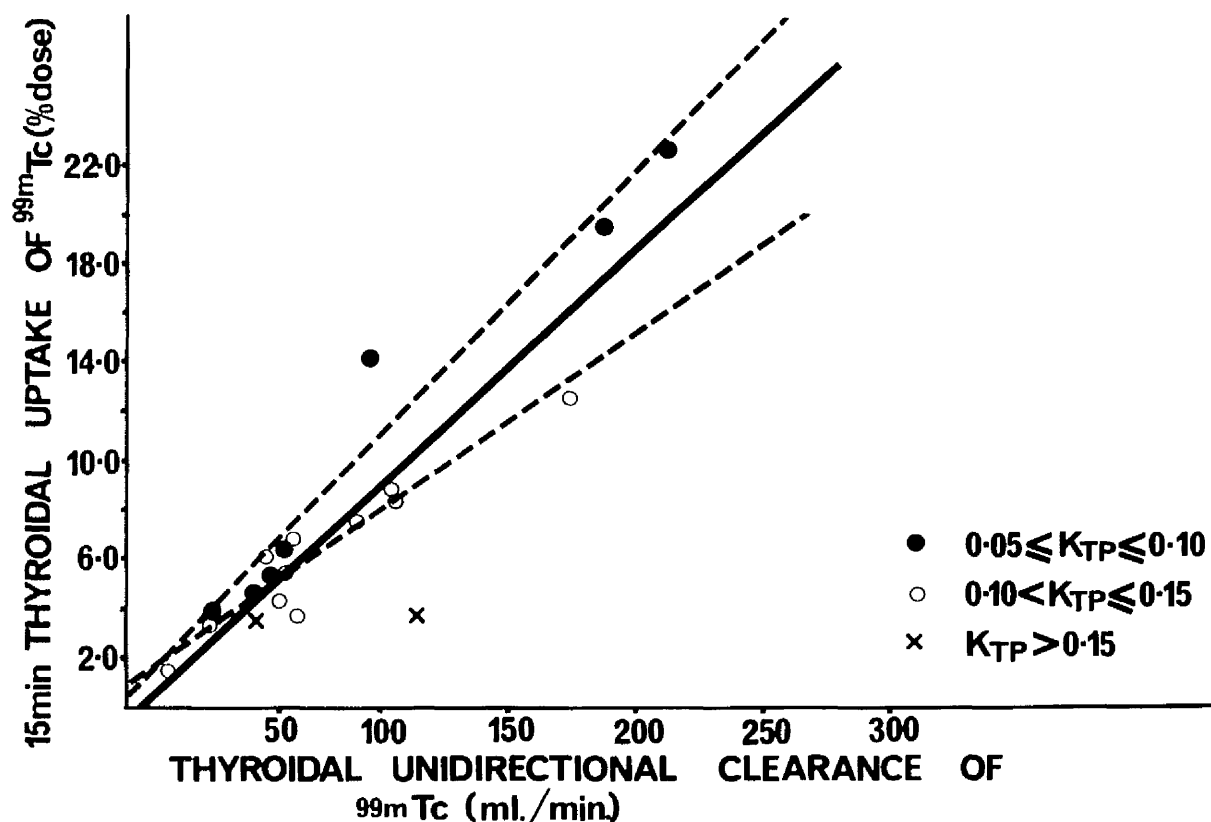


Figure 25: This figure shows the correlation between thyroidal uptake and unidirectional clearance of pertechnetate- ^{99m}Tc measured in 20 patients. The upper broken regression line, $y = 0.107x + 0.19$ and $r = 0.98$, relates to 7 patients (closed circles) with $0.05 \leq K_{TP} \leq 0.10$ and the lower broken regression line, $y = 0.0695x + 0.96$ and $r = 0.95$ relates to 11 patients (open circles) with $0.10 < K_{TP} \leq 0.15$. Two patients are shown where $K_{TP} > 0.15$. The unbroken regression line for all patients was $y = 0.095x - 0.46$ with $r = 0.90$.

TABLE 7

THE RELATIONSHIP BETWEEN THYROID UPTAKE,
UNIDIRECTIONAL CLEARANCE AND EXIT-RATE CONSTANT
OF ^{99m}Tc IN EUTHYROID AND THYROTOXIC SUBJECTS

PATIENT	TECHNETIUM-99m		
	THYROID UNIDIRECTIONAL CLEARANCE (L/min)	15 MINUTE THYROID UPTAKE % DOSE	THYROID K_{TP} (min ⁻¹)
1	0.230	22.7	0.083
2	0.056	5.1	0.094
3	0.056	4.6	0.080
4	0.100	14.0	0.053
5	0.032	3.8	0.095
6	0.072	6.2	0.080
7	0.265	19.5	0.095
8	0.055	6.0	0.105
9	0.105	8.8	0.105
10	0.060	4.2	0.150
11	0.130	8.9	0.140
12	0.064	5.4	0.130
13	0.019	1.5	0.105
14	0.066	6.7	0.110
15	0.175	12.5	0.150
16	0.090	7.5	0.105
17	0.034	3.4	0.140
18	0.068	3.8	0.125
19	0.115	3.8	0.26
20	0.052	3.5	0.29

RESULTS

Uptake of ^{99m}Tc by the thyroid, 15 minutes after injection in the 6 groups of patients, is shown in Figures 23 and 24. Results are summarised in Table 6.

The relationship between thyroid unidirectional clearance and the 15 minute uptake of ^{99m}Tc in the 4 euthyroid and 16 thyrotoxic patients is shown in Figure 25 and the complete data in Table 7. The regression line for 7 patients with $0.05 \leq K_{\text{TP}} \leq 0.10$ was $y = 0.107 x + 0.19$ with $r = 0.978$. The regression line for 11 patients with $0.10 < K_{\text{TP}} \leq 0.15$ was $y = 0.0695 x + 0.96$ with $r = 0.95$. The regression line for all 20 patients studied was $y = 0.095 x - 0.46$ with $r = 0.90$.

Plasma ^{99m}Tc concentration at 15 minutes in 4 euthyroid patients, was 0.091 ± 0.019 (mean \pm 1SD) and in 16 thyrotoxic patients, was 0.087 ± 0.016 (mean \pm 1SD) fract. dose/litre.

DISCUSSION

This directional counting technique for precise measurement of thyroid uptake of ^{99m}Tc (strictly the 'washout' of thyroidal ^{99m}Tc) has the advantage of technical simplicity over quantitative scanning procedures. Moreover, separate measurement of ETA is not required and so a major source of error is eliminated. The resultant index is both reproducible and sensitive enough to be measured in hypothyroid patients. Ranges for normal and diseased subjects appear to be similar to those reported by other workers (Goolden et al, 1971; McGill et al, 1971) who used quantitative scintiscanning, despite the

small systematic error of non-dischargeable ^{99m}Tc . In clinical terms it appears that non-dischargeable ^{99m}Tc is unimportant.

The close collimation used to reduce the contribution of ETA to total neck counts restricts application of the technique to thyroid glands under 100 g. This minor disadvantage is rarely a problem in practice.

There are two main disadvantages of the protocol. Firstly, administration of perchlorate and resultant blocking of thyroidal iodide transport will delay additional dynamic studies of thyroid function for at least 24 hours. Secondly, intravenous injection of ^{99m}Tc and perchlorate is necessary because use of the more convenient oral route is accompanied by the major systematic error of variable alimentary absorption (Alexander et al, 1969).

The ^{99m}Tc uptake is clearly a resultant of two dynamic processes: ^{99m}Tc influx into and efflux from, thyroid. Since thyroid and plasma ^{99m}Tc pools are in equilibrium at 15 minutes in the majority of patients,

$$C.P(ti) = K_{TP} T(ti)$$

where C = thyroid unidirectional clearance of ^{99m}Tc

P(ti) = plasma concentration of ^{99m}Tc at 15 min

K_{TP} = exit-rate constant of thyroid for ^{99m}Tc

T(ti) = thyroid content of ^{99m}Tc at 15 min

Therefore:

$$T(ti) = \frac{C.P(ti)}{K_{TP}} \quad \text{where } ti = 15 \text{ min}$$

The thyroid uptake of ^{99m}Tc at equilibrium is therefore directly

proportional to both unidirectional clearance and ambient plasma concentration of ^{99m}Tc and inversely proportional to K_{TP} . $P(\text{ti})$, which is mainly dependent on plasma volume, may also be affected by a variable thyroid unidirectional clearance C . K_{TP} also varies between patients though 90% of subjects had K_{TP} values between 0.05 and 0.15 min^{-1} . Ignoring the variability of plasma ^{99m}Tc , however, it is clear that the relationship between uptake ($T(\text{ti})$) and unidirectional clearance (C) cannot be linear and any regression line obtained between these parameters will depend in part on K_{TP} values in the sample population studied (Figure 25). Since a majority of patients had K_{TP} values within a small range, the error in estimating unidirectional clearance from the uptake in any one patient is unlikely to be great if the regression line for the whole population is used. This particular inaccuracy does indicate, however, that the uptake measurement will be most valuable for sequential assessment of iodide transport in the same patient where K_{TP} and possibly $P(\text{ti})$, may be assumed constant.

The results have confirmed reports of other workers (Goolden et al, 1971; McGill et al, 1971; Van't Hoff et al, 1972) that thyroid uptake of ^{99m}Tc is of value for diagnosis of thyrotoxicosis. Uptake of ^{99m}Tc may overlap into the thyrotoxic range, however, when other thyroid pathology with hyperactive iodide transport is present (e.g. Hashimoto's disease).

There are specific situations where use of ^{99m}Tc is preferable to that of radioactive iodine. In view of the small radiation dose to

thyroid and whole body (De Garreta et al, 1968) and more suitable counting characteristics, it should replace ^{132}I for measurements of thyroid uptake in children and pregnant women. Since thyroid organification of radionuclide is minimal, $^{99\text{m}}\text{Tc}$ kinetics are not influenced by concurrent administration of thiourylene drugs such as carbimazole. $^{99\text{m}}\text{Tc}$ uptake of thyroid can therefore be used to follow the progress of thyrotoxic patients on drug treatment (Goolden et al, 1971) and can even indicate thyroid suppressibility (Shimmins et al, 1971).

In conclusion, the technique described provides a useful simple alternative to quantitative scintiscanning and scintillation-camera procedures when measurements of the thyroid uptake of $^{99\text{m}}\text{Tc}$ are thought to be useful in clinical diagnosis and research.

SUMMARY - CHAPTER 9

The 15 minute uptake of ^{99m}Tc by thyroid was measured using a simple directional counting technique whereby the washout of thyroidal ^{99m}Tc was quantitated after intravenous perchlorate injection. Results from patients with various thyroid disorders compared favourably with those obtained by other workers who used quantitative scintiscanning and scintillation camera techniques. Thyroid uptake of ^{99m}Tc was found to be largely proportional to the unidirectional clearance of ^{99m}Tc though an error in this relationship resulted from inter-patient variability of thyroidal K_{TP} . It is suggested that the role of ^{99m}Tc uptake in thyroid investigation should be primarily in sequential assessment of thyroidal iodide transport in the same patient where K_{TP} may be assumed constant.

CHAPTER 10

EFFECT OF RADIOACTIVE IODINE THERAPY ON THE ACTIVITY OF IODIDE TRANSPORT AS MEASURED BY THYROID UPTAKE OF TECHNETIUM-99m

INTRODUCTION

A vast literature on radioactive iodine-131 (^{131}I) therapy for thyrotoxicosis spanning two decades reflects the difficulties in providing a permanent remission of thyrotoxicosis with this treatment without a cumulative rising incidence of iatrogenic hypothyroidism with passage of time. Workers have attempted to predict the eventual outcome of ^{131}I therapy by performing in vivo thyroid function tests using tracer amounts of ^{131}I (Soley et al, 1949; Larson, 1955) and ^{132}I (Einhorn et al, 1961; Binopoulos et al, 1963; Buchanan et al, 1963) at short intervals after treatment. Although the results of such studies are instructive and demonstrate the effect of radiation therapy on aspects of thyroid function, therapeutic success has been limited by the variability in thyroid radiosensitivity from patient to patient.

Greig et al (1969) have recently pioneered the use of radioactive iodine-125 (^{125}I) as an alternative therapeutic agent. The initial hypothesis (Gillespie et al, 1970) that low energy B irradiation from ^{125}I would affect hormone synthesis more than cell replication has been confirmed in animal studies (Gross et al, 1968; Greig et al, 1970). Prolonged clinical trials are at present in progress to determine whether the unusual microdosimetry of ^{125}I will reduce the cumulative

incidence of post-therapy hypothyroidism (McDougall et al, 1971; Werner et al, 1970; Bromner et al, 1973).

The effect of ^{125}I treatment on thyroidal iodide transport has not been studied in detail (Lewitus et al, 1971). Thyroid uptake of $^{99\text{m}}\text{Tc}$ was therefore measured 15 minutes after injection, in thyrotoxic patients before, and at intervals after, treatment with ^{131}I or ^{125}I . Since the photon energy of $^{99\text{m}}\text{Tc}$ (140 KeV) is higher than that of ^{125}I (25 - 35 KeV), studies were possible immediately following ^{125}I therapy. Similar early studies following ^{131}I therapy were precluded by the higher energy (360 KeV) of this radionuclide and in this group of patients, assessment began 1 - 2 months after therapy. Results on 33 treated thyrotoxic patients are presented and correlated with the clinical outcome 8 - 10 months later.

MATERIALS AND METHODS

Thirty-three patients were studied and in all, a clinical diagnosis of thyrotoxicosis was confirmed by a 24 hour uptake of ^{131}I and serum PB^{127}I . All patients were treated as out-patients and each had diffuse thyroid enlargement with an approximate thyroid weight of between 25 and 75 g. The therapy dose of ^{131}I or ^{125}I , administered orally, contained approximately 60 ng $^{127}\text{I}^-$ and was calculated on an empirical basis depending on approximate gland weight. Since qualitative data on the radiation effect was required, estimates of radiation dose to each gland were not made. Thirteen female patients, with a mean age of 46 years (range of 39 - 62 years), were given $7 \pm 4 \text{ mCi } ^{131}\text{I}$ (mean \pm 1SD) in 18 therapy doses. Twenty patients, 2 male and 18 female with a mean age of 45 years (range of 36 - 54

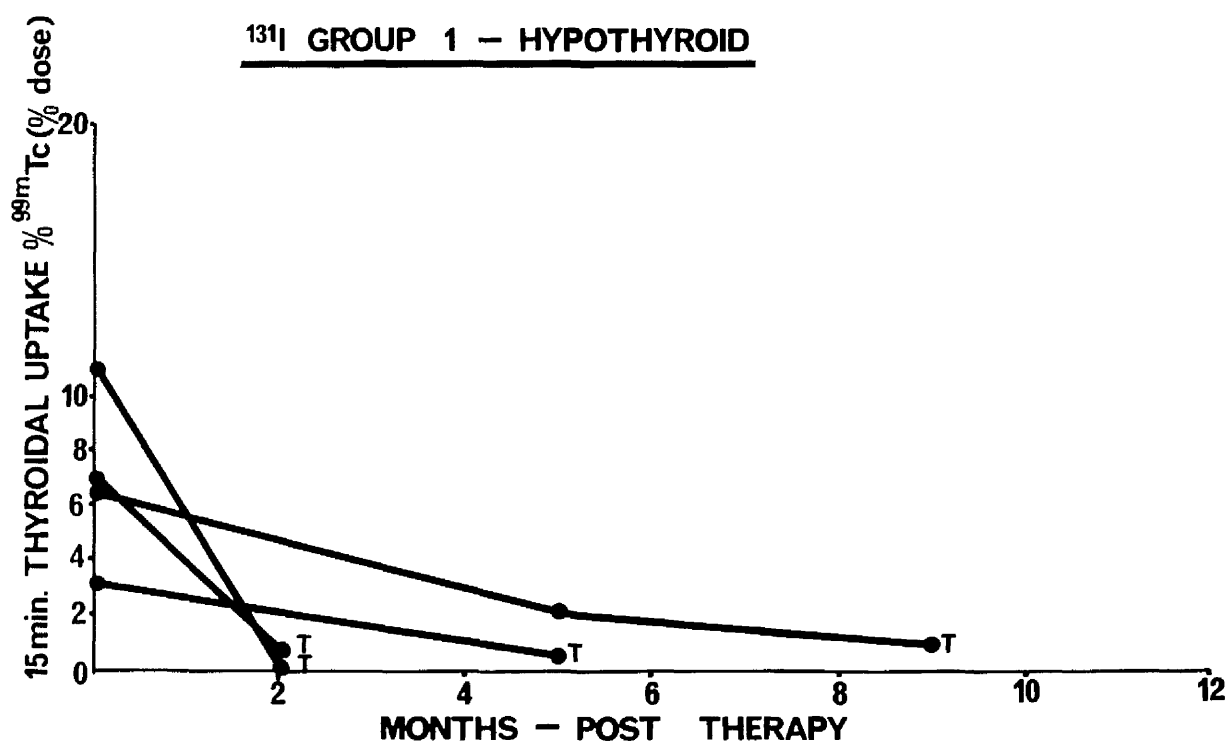


Figure 26: Thyroid uptake of ^{99m}Tc measured sequentially in 4 patients who became hypothyroid after ¹³¹I therapy. 'T' indicates commencement of thyroxine.

years), were given 12 ± 4 mCi ^{125}I (mean \pm 1SD) in 21 therapy doses.

Thyroid function was measured with a serum FB ^{127}I and thyroid uptake of $^{99\text{m}}\text{Tc}$ (Chapter 9) before and regularly after treatment. Assessment began 2 - 4 days after ^{125}I and 1 - 2 months after ^{131}I therapy.

Thyroid content of therapy ^{125}I following treatment was measured at weekly intervals for 4 - 6 weeks in 12 patients using an adapted directional counting system. This consisted of a standard IAEA collimator with a 1.0 mm thick copper sheet at a standard distance between the crystal and patient to reduce intensity of radiation from treated glands. Suitable therapy dose standards of ^{125}I in a thyroid phantom were used for system calibration.

The biological half-life of thyroidal ^{125}I was calculated by plotting the fraction of 24 hour ^{125}I thyroid uptake which remained in the gland on a log-linear scale with time. The time required for thyroid ^{125}I content to fall to 50% of the 24 hour uptake was considered to be the biological half-life ($T_{\frac{1}{2}} \text{ Biol}$).

RESULTS

Data from ^{131}I and ^{125}I treatment populations has been grouped depending on the clinical outcome of therapy at 8 - 10 months after treatment. Variation in thyroid uptake of $^{99\text{m}}\text{Tc}$ with time after therapy is shown in Figs. 26 to 31 and the data is summarised in Figs. 27b to 30b.

^{131}I THERAPY

Group 1

Hypothyroid result: (Fig. 26). Two patients with a

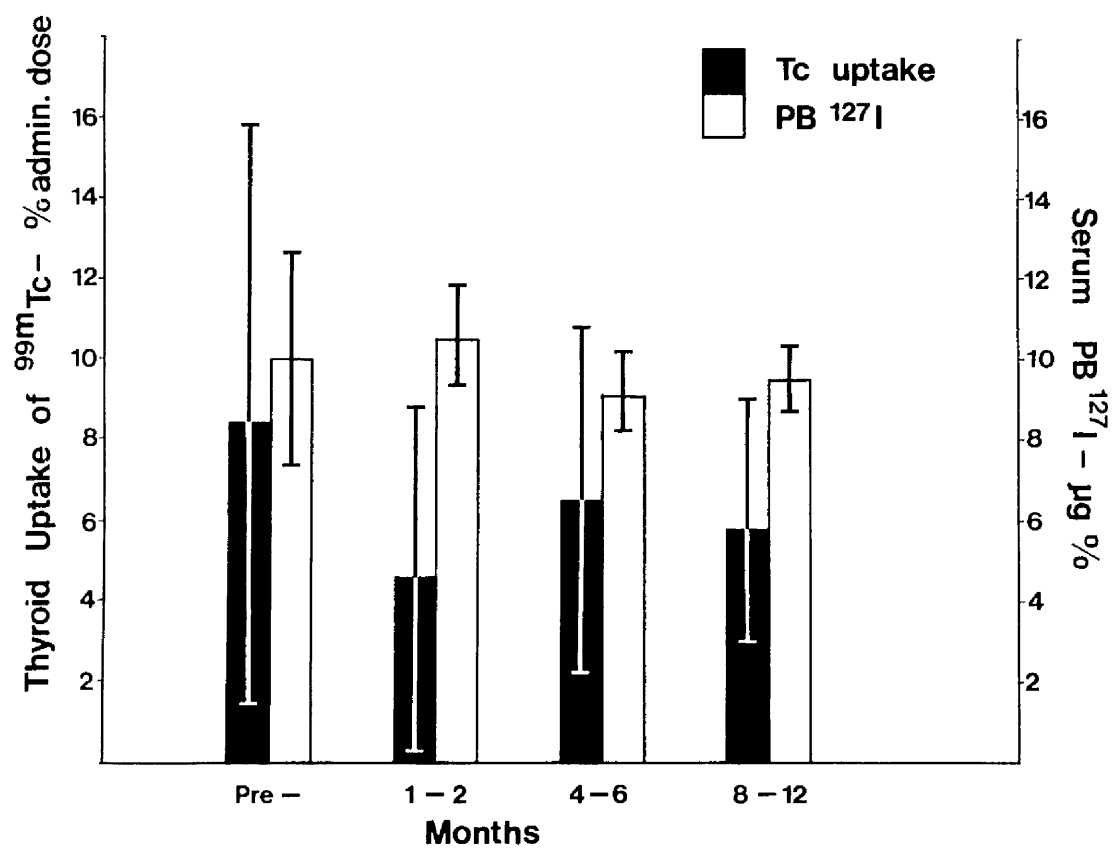
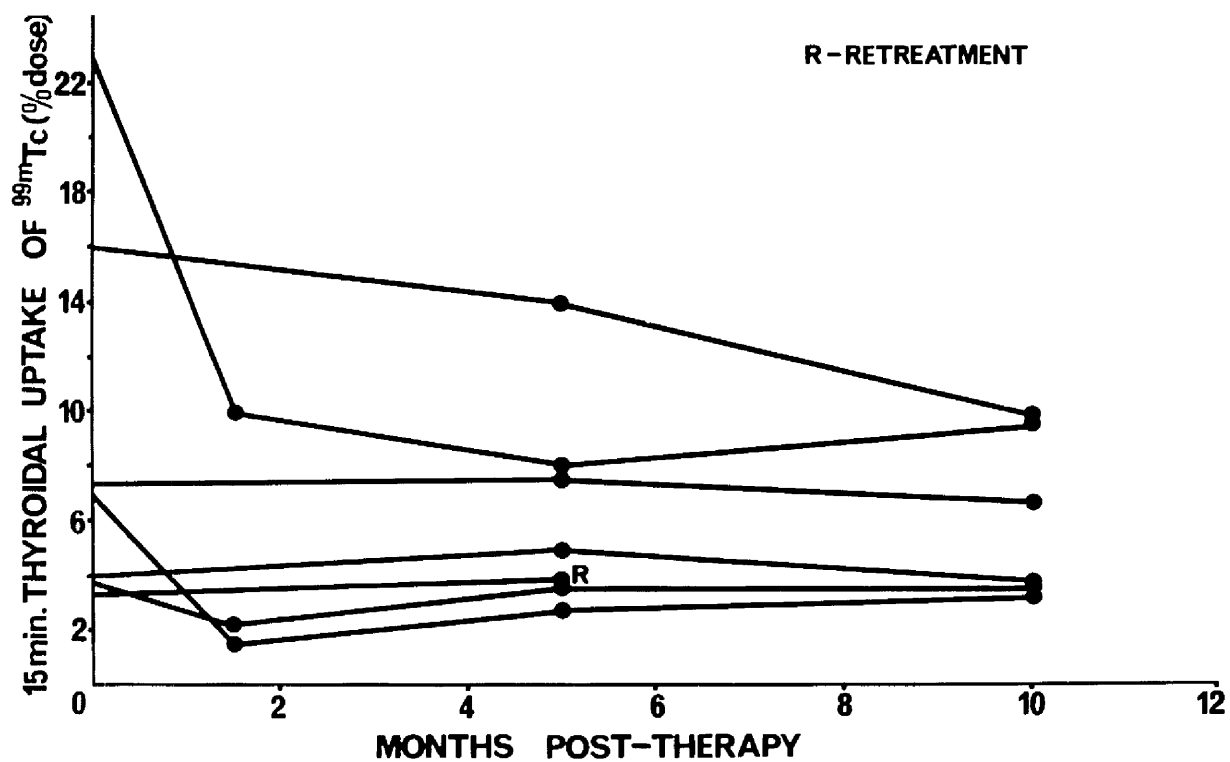


Figure 27a: Thyroid uptake of ^{99m}Tc measured sequentially after ^{131}I therapy in 7 patients who remained thyrotoxic.

Figure 27b: Summary of sequential assessment of thyroid function in the 7 patients who remained thyrotoxic after ^{131}I therapy. The mean \pm LSD of each parameter in the patient group is illustrated.

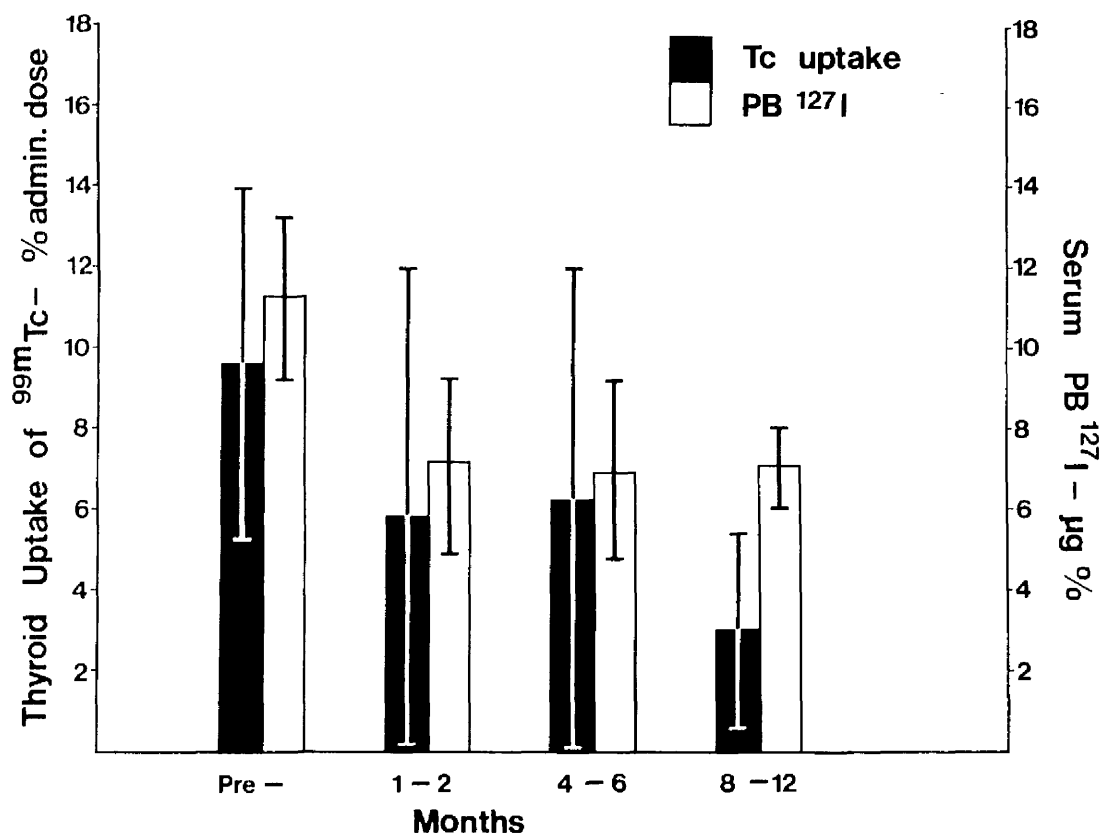
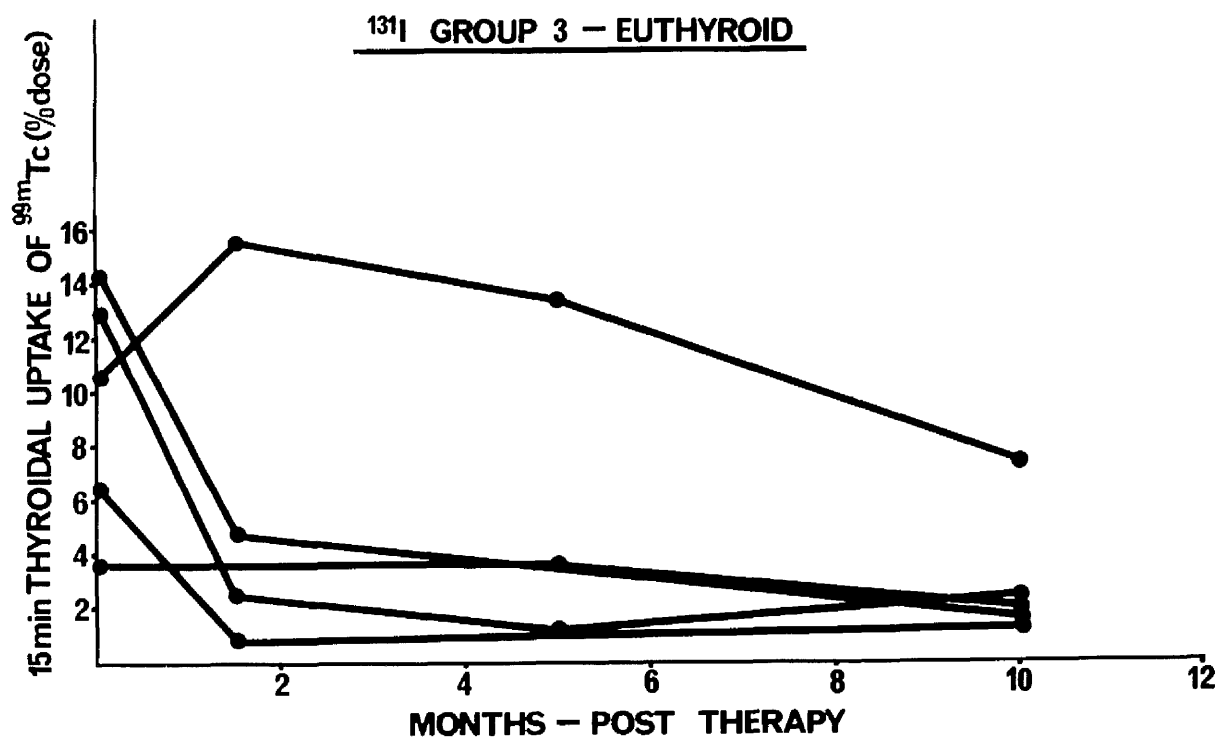


Figure 28a: Thyroid uptake of ^{99m}Tc measured sequentially after ^{131}I therapy in 5 patients who became euthyroid. The patient whose uptake increased after therapy had a major defect of iodide organification in thyroid and was clinically and biochemically euthyroid throughout this period of time.

Figure 28b: Summary of sequential assessment of thyroid function in 5 patients who became euthyroid after ^{131}I therapy. The mean \pm LSD of each parameter in this patient group is illustrated.

precipitate fall in ^{99m}Tc uptake developed hypothyroidism within 2 months. In 2 other patients, an apparent gradual fall of ^{99m}Tc uptake over 5 - 9 months resulted in hypothyroidism.

Group 2

Persistent thyrotoxicosis: Fig. 27a shows the following features:

1. In 3 patients, ^{99m}Tc uptake fell to 40 - 70% of pre-treatment value within 2 months. ^{99m}Tc uptake remained at the new level in 2 while in the other, the pre-treatment level was re-attained within 5 months.
2. In 3 patients there was little change in ^{99m}Tc uptake following therapy while in 1 other, the uptake gradually fell to 60% pre-treatment value at 10 months.

Fig. 27b, which summarises the data, demonstrates a fall in mean ^{99m}Tc uptake at 2 months with rise thereafter. The mean PB ^{127}I was virtually unchanged over the period of observation.

Group 3

Euthyroid result: Fig. 28a shows the following features:

1. In 3 patients, ^{99m}Tc uptake fell to 15 - 30% of pre-treated value within 2 months. Each patient was euthyroid at this time and was still euthyroid with little change in ^{99m}Tc uptake 10 months after treatment.
2. One patient was seen to be euthyroid 5 months after therapy with no change in ^{99m}Tc uptake. The uptake fell slightly at 10 months.

¹²⁵I GROUP 4 EUTHYROID

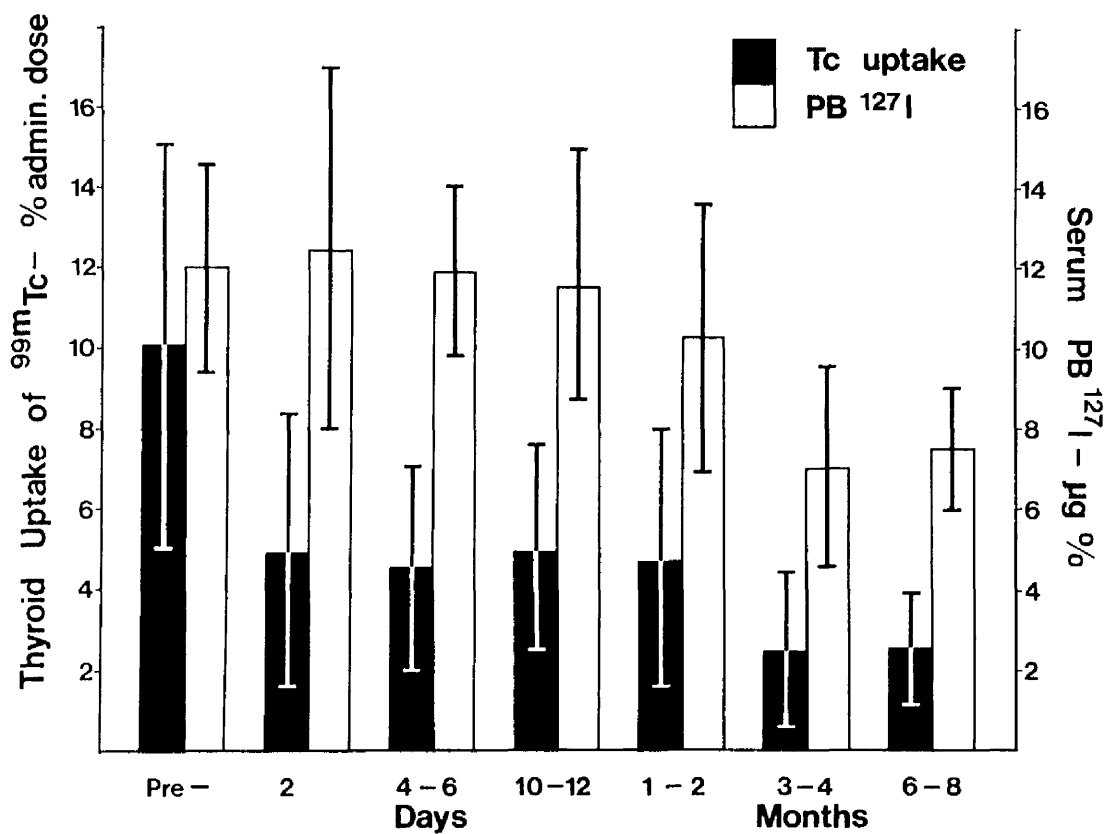
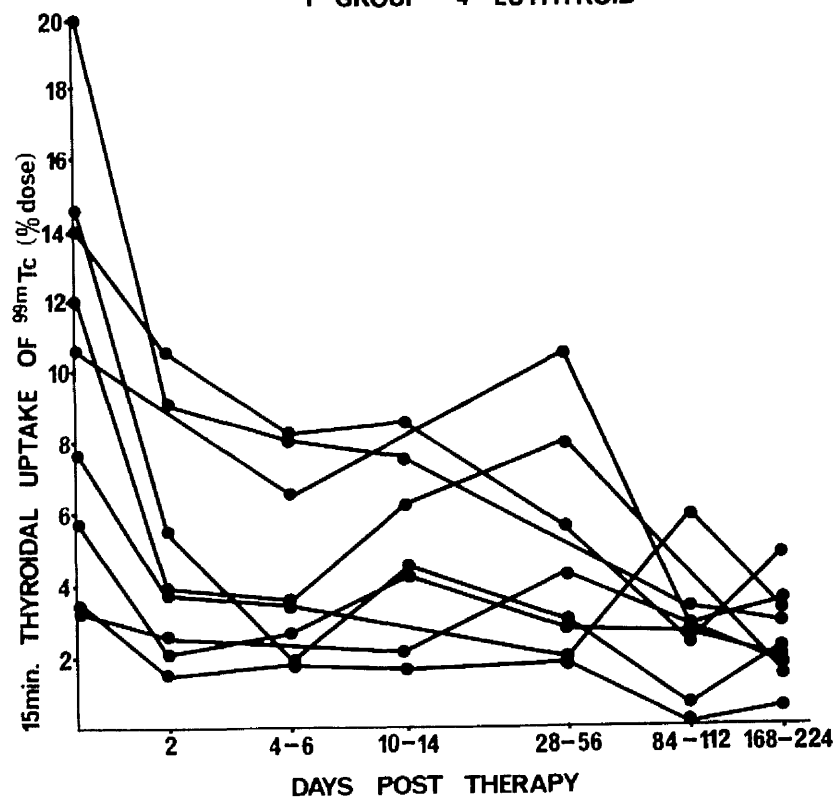


Figure 29a: Thyroid uptake of ^{99m}Tc measured sequentially after ^{125}I therapy in 9 patients who became euthyroid.

Figure 29b: Summary of sequential assessment of thyroid function in 9 patients who became euthyroid after ^{125}I therapy. The mean \pm LSD of each parameter in this patient group is illustrated.

3. One patient demonstrated a rise in ^{99m}Tc uptake at 2 months after therapy despite the presence of clinical euthyroidism at this time. The uptake then fell gradually until it approximated the pre-treatment level at 10 months. This discrepancy between uptake and clinical state was accounted for by a demonstrable defect of thyroidal iodide organification (Chapter 13).

Fig. 28b, which summarises the data, demonstrates a fall in both ^{99m}Tc uptake and PB^{127}I by 2 - 4 months and the relative constancy of PB^{127}I thereafter.

^{125}I THERAPY

Group 4

Euthyroid result: Fig. 29a shows the following notable features:

1. In all patients studied, there was a reduction in ^{99m}Tc uptake at 2 - 4 days to 40 - 70% of pre-treatment value.
2. In 3 patients, ^{99m}Tc uptake continued to fall with time. By 8 months it stabilised at 10 - 30% of the pre-treatment value.
3. In 6 patients, ^{99m}Tc uptake increased between 2 - 8 weeks then fell once more, stabilising at 10 - 40% of the pre-treatment value at 8 months.

Fig. 29b, which summarises the data, demonstrates an initial precipitate fall in ^{99m}Tc uptake on the second day and further reduction over succeeding months. PB^{127}I shows a delayed fall.

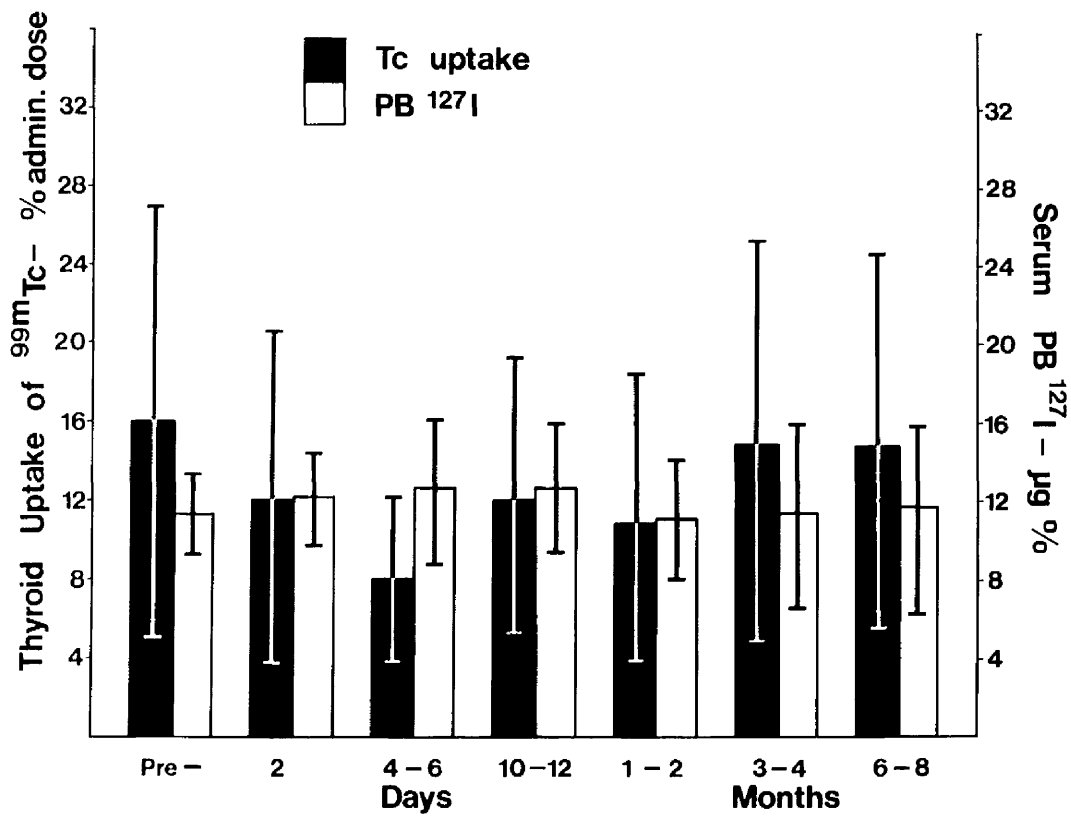
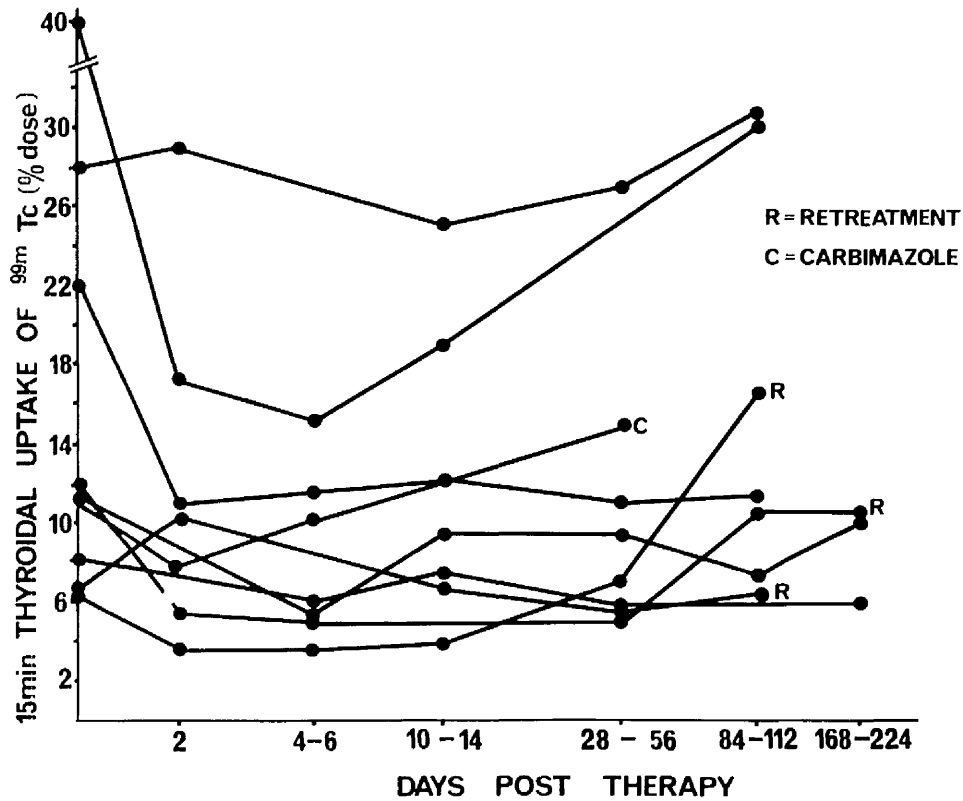


Figure 30a: Thyroid uptake of ^{99m}Tc measured sequentially after ^{125}I therapy in 9 patients who remained thyrotoxic.

Figure 30b: Summary of sequential assessment of thyroid function in 9 patients who remained thyrotoxic after ^{125}I therapy. The mean \pm LSD of each parameter in this patient group is illustrated.

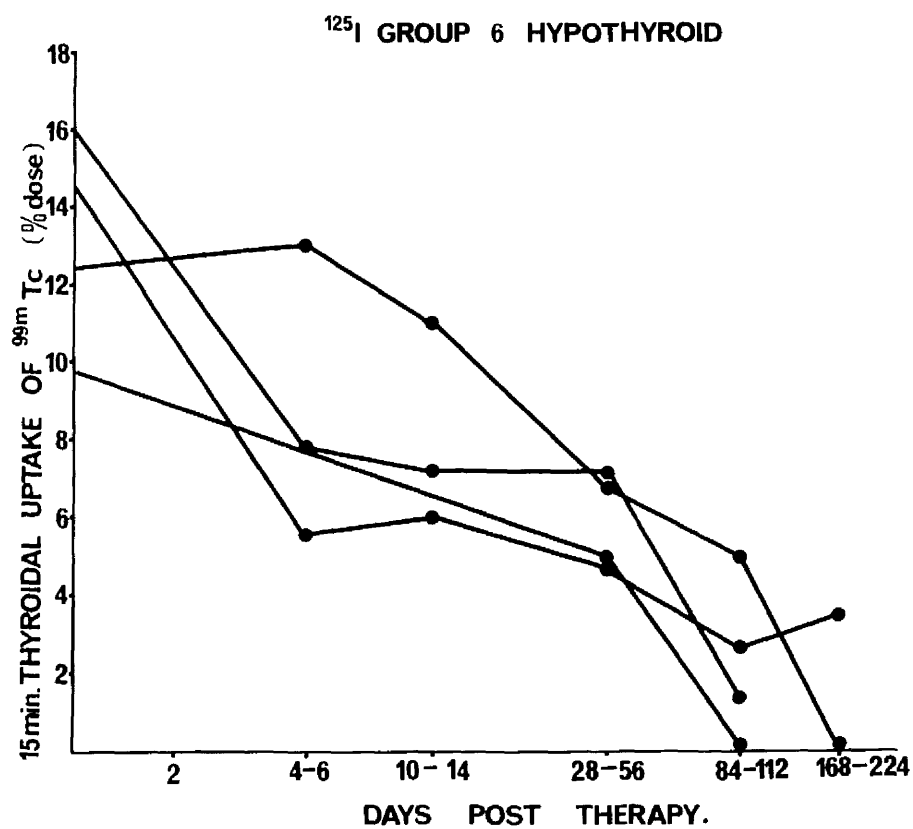


Figure 31: Thyroid uptake of ^{99m}Tc measured sequentially after ¹²⁵I therapy in 4 patients who became hypothyroid.

Group 5

Persistent thyrotoxicosis: Fig. 30a shows the following notable features:

1. Three patients showed little change in ^{99m}Tc uptake following therapy.
2. In 6 patients, there was a fall in ^{99m}Tc uptake within 2 - 4 days to 50 - 70% of the pre-treatment value. In 4 of these patients, uptake rose after 6 days and reached 75 - 90% of the pre-treatment value by 4 months. In 1 patient, the uptake persisted at the value attained 2 days post-therapy and in the other patient it rose to 250% of the pre-treatment value by 4 months.

Fig. 30b, which summarises the data, demonstrates little change in mean PB^{127}I with time though a modest reduction in mean ^{99m}Tc uptake is seen at early intervals following treatment.

Group 6

Hypothyroid result: Fig. 31a shows the following notable features:

1. In 2 patients, the initial fall in ^{99m}Tc uptake to 50% of the pre-treatment value within 4 days was maintained for 1 - 2 months. Thereafter, 1 patient became hypothyroid within 4 months with a further fall in ^{99m}Tc uptake and the other within 10 months.
2. In 1 patient, the ^{99m}Tc uptake fell only from the tenth day post-therapy. Thereafter, the uptake fell gradually till hypothyroidism developed at 8 months.

BIOLOGICAL HALF-LIFE OF THYROIDAL ^{125}I

The biological half-life of thyroidal ^{125}I in 12 patients was

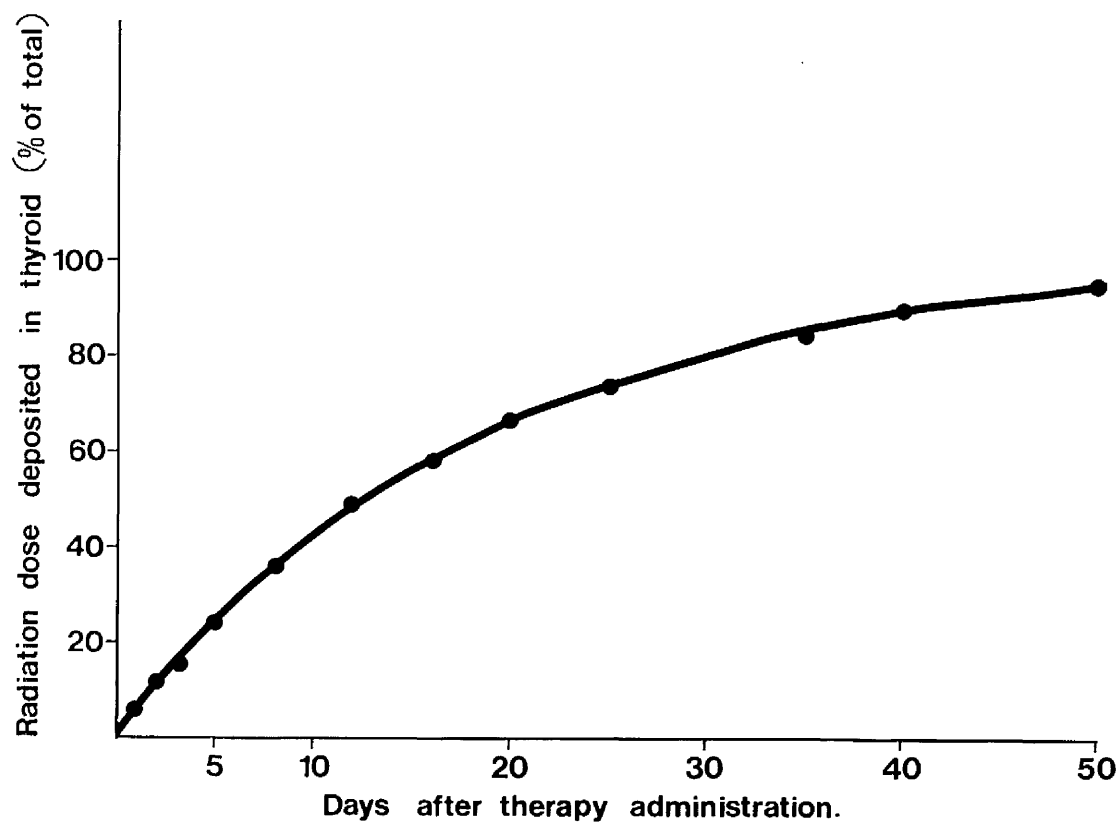


Figure 32: This figure illustrates the progressive increase in total radiation exposure of thyroid from an average therapy dose of ^{125}I . Approximately 11% of total radiation is deposited in 2 days and 50% in 13 days.

16 days \pm 7 (1SD). The derived effective half-life ($T_{1/2}$ eff) was 12.6 days (Appendix 3).

Using the calculated $T_{1/2}$ eff with the mean 24 hour uptake of ^{125}I therapy dose in the 12 patients (i.e. 68%), the average increase in total radiation exposure of thyroid with time can be calculated (Appendix 3) and has been portrayed in graphical form (Figure 32).

It can be seen that, on average, 50% of total radiation is deposited in 13 days and at 50 days, 95% of exposure is complete.

DISCUSSION

Workers who studied thyroid function in vivo following ^{131}I therapy (Larson and Raghult, 1953; Myant, 1953; Einhorn and Hastad, 1961; Binopoulos et al, 1963) clearly demonstrated that thyroid uptakes of ^{131}I , 3 weeks after therapy, were reduced from pre-treatment levels in most cases. Although reduction in ^{131}I uptake at this time was most pronounced in the group of patients eventually becoming euthyroid or hypothyroid, attempts to predict the clinical outcome in individual patients by graduating this reduction in uptake were unsuccessful. Indeed 2 - 3 months after therapy, the uptake usually rose to pre-therapy levels in patients requiring re-treatment and remained at a low level or became lower in patients eventually becoming euthyroid or hypothyroid.

In this investigation of patients after ^{131}I therapy the author has confirmed firstly that reduction in $^{99\text{m}}\text{Tc}$ uptake at 6 - 8 weeks after therapy is greater in those becoming euthyroid or hypothyroid, and secondly that prediction of clinical outcome is inaccurate where

individual patients are concerned unless studies are performed 4 - 6 months after therapy. An interesting feature has been the elevated ^{99m}Tc uptake after therapy in the patient who developed a defect of thyroidal iodide organification. This particular radiation-induced dysshormonogenesis will be discussed fully in Chapter 14.

Data from patients following ^{125}I therapy are more valuable and allow closer insight into ^{the}effect of this radiation on thyroid function. Three main features emerge. Firstly, an appreciable reduction in ^{99m}Tc uptake observed 2 days after therapy in most patients indicates an immediate radiation effect on follicular function. As depicted in Figure 32, only a mean 11% of the total radiation dose to thyroid would have been deposited at this time. It seems plausible that this initial effect on thyroid function is related more to the therapy dose-rate than to total dose delivered at that time. Secondly, ^{99m}Tc uptake appears to increase 6 - 12 days after therapy; while it may return to pre-therapy levels in those requiring re-treatment, it usually decreases again in those becoming euthyroid. It is probable that this second decrease in ^{99m}Tc uptake is primarily related to the total radiation dose delivered to thyroid. Thirdly, a slow reduction in ^{99m}Tc uptake over 2 - 6 months after therapy in those patients becoming hypothyroid is clearly indistinguishable in the early stages from those destined to remain euthyroid.

These results suggest that after 2 months, the effect of ^{125}I radiation on thyroidal iodide transport, closely parallels that of ^{131}I . Following therapy with either radionuclide, one clearly must

wait 4 - 6 months before a ^{99m}Tc uptake or PB ^{127}I will reliably indicate residual function in the thyroid remnant. The observation that PB ^{127}I measurement is valueless for predicting clinical outcome at early intervals after therapy was first made by Larson (1955) and reflects the slow turnover of total body thyroxine.

The results of this pilot study have enabled the author to formulate a simple hypothesis for the mechanism of action of ^{125}I radiation on thyroidal iodide transport and possibly on total thyroid function. In essence, the immediate effect of this radiation would appear to be a reduction of iodide transport across the basal membrane and may be termed 'functional radiosensitivity'. This is possibly dose-rate related and mainly reversible. The delayed, cumulative effect on iodide transport may be related to total radiation deposition in the gland and will probably be irreversible. The net result of this latter effect will be 'radio-destruction' of cells and reduction in the functional cell mass of thyroid. The observation that one patient showed little evidence of functional radiosensitivity, yet later developed hypothyroidism (Figure 31:) might indicate that functional radio-sensitivity and radio-destruction are potentially distinct responses of thyroid to radiation. Further animal and human in vivo studies are required to clarify these points.

From the practical aspect, it is clear that a combination of PB ^{127}I and ^{99m}Tc uptake at 4 months after therapy will reliably indicate the eventual clinical outcome in the majority of patients. While not ideal, this will be of practical importance when attempts are

made to reduce the incidence of post ^{125}I therapy hypothyroidism by administration of fractionated therapy doses rather than the single doses currently employed. Further studies are clearly required to determine whether fractionation of ^{125}I therapy will control the intractable problem of hypothyroidism after treatment.

SUMMARY - CHAPTER 10

Thyroid uptake of ^{99m}Tc , 15 minutes after injection, was measured in thyrotoxic patients before, and at intervals after, treatment with ^{125}I or ^{131}I . In a detailed study, the radiobiological effect of ^{125}I on thyroidal iodide transport was found to be biphasic with an immediate, reversible effect possibly related to rate of delivery of treatment radioactivity and a delayed, irreversible effect, possibly related to total deposited radioactivity. The results argue that measurement of ^{99m}Tc uptake 4 months after therapy will give a reliable indication of treatment effectiveness in a majority of patients.

SECTION 4

STUDIES OF THYROIDAL IODIDE ORGANIFICATION

IN VIVO USING RADIOACTIVE IODINE-131

- | | |
|------------|---|
| Chapter 11 | The intravenous perchlorate discharge test:
theoretical aspects, technical development
and clinical application |
| Chapter 12 | The organification rate of thyroidal radio-
active iodide-131: measurement in vivo |
| Chapter 13 | Hashimoto's disease: its differentiation
from simple goitre using the intravenous
perchlorate discharge test |
| Chapter 14 | Defective iodide organification in thyroid
following therapeutic irradiation with
radioactive iodine-131 or 125 |
| Chapter 15 | Familial goitrous hypothyroidism:
investigation of three siblings |

CHAPTER 11

THE INTRAVENOUS PERCHLORATE DISCHARGE TEST:

THEORETICAL ASPECTS, TECHNICAL DEVELOPMENT

AND CLINICAL APPLICATION

In this chapter, a simple in vivo technique for detection of unorganified radioactive iodide in thyroid is developed and assessed with the aid of small-animal studies.

INTRODUCTION

Detection of unorganified thyroidal iodide with the conventional oral perchlorate discharge test assists diagnosis of conditions such as Hashimoto's disease, Pendred's syndrome and iodide goitre, where a defect of iodide organification is present. In the conventional test, potassium perchlorate is given orally in a dose of up to one gram, up to 2 hours after oral administration of radioactive iodine- ^{131}I to the patient. Thyroid content of ^{131}I is monitored by directional counting and, if unorganified iodide is present, a discharge of inorganic ^{131}I from the gland is seen after perchlorate administration. The insensitivity of this standard test, however, makes detection of small amounts of thyroidal inorganic iodide unreliable and this feature is reflected in a lack of unanimity among protagonists of the oral test regarding methodology and criteria for its interpretation (Fraser et al, 1960; Floyd et al, 1960; Baschieri et al, 1963; Stewart and Murray, 1966).

A discharge test has been developed using intravenous perchlorate:

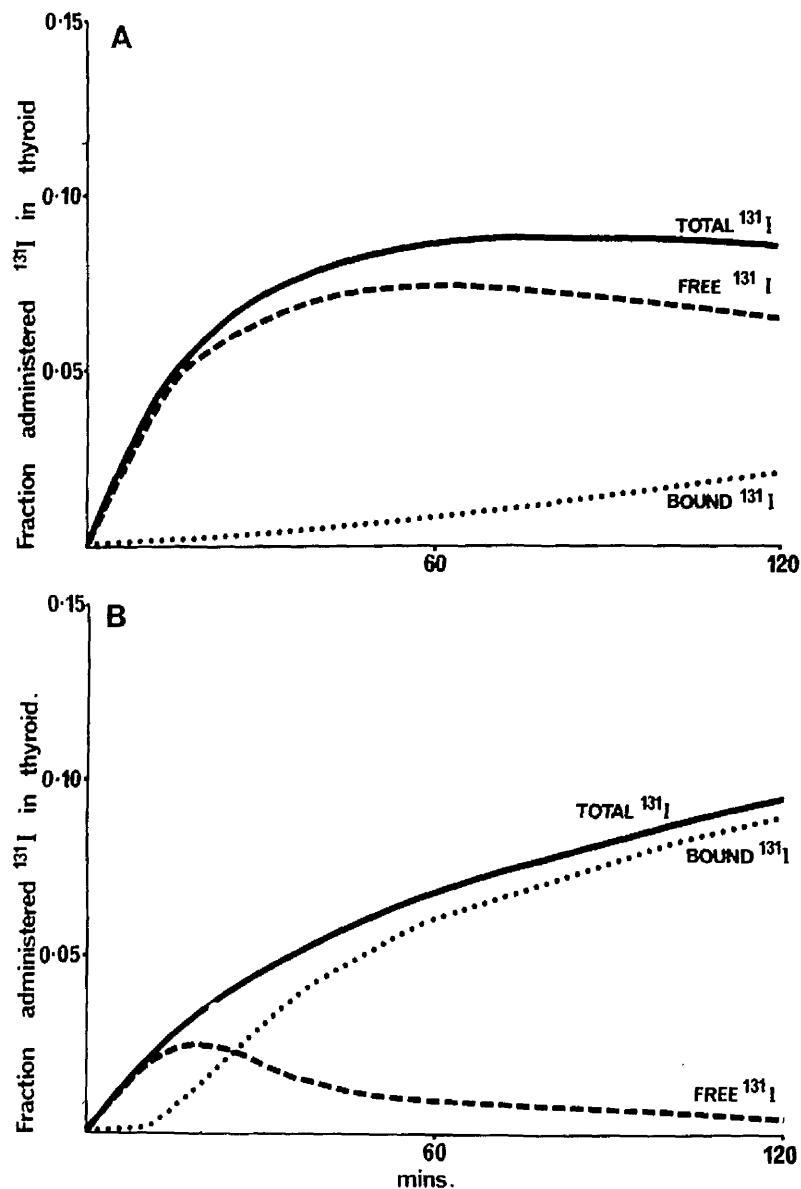


Figure 33: Hypothetical representation of the partition of IV ^{131}I into organic and inorganic thyroid pools as a consequence of the three-compartment model.

- (A) Major defect of iodide organification:- with organification rate of approximately zero, there is a large persistent inorganic ^{131}I pool in thyroid.
- (B) Minor defect of iodide organification:- with organification rate approximately 0.20, the large inorganic ^{131}I pool in thyroid present initially diminishes rapidly in size with time.

it is simpler, more rapidly performed and possibly more sensitive than the oral test. Results obtained using this new test in patients with various thyroid disorders are contrasted ^{with} / data from rats which were studied using a similarly conceived discharge technique.

BASIC KINETICS

As described in Chapter 4, thyroid transport of plasma iodide and its subsequent organification in the gland can be considered theoretically in terms of a three compartment model (Berson and Yalow, 1955; Wollman and Reed, 1959; Robertson et al, 1971). Basically, thyroid unidirectional clearance of plasma iodide (the trap) pulls iodide into the gland and the fraction of this trapped iodide which is organified depends on the organification rate. With normal iodide organification, no unorganified thyroid iodide can be demonstrated (Berson and Yalow, 1955) and the organification rate approximates unity. With a reduction in organification rate, less trapped iodide is organified and more accumulates as inorganic thyroidal iodide.

Correct timing of perchlorate administration is important in performing a discharge test. Hypothetical thyroid uptake patterns of ^{131}I following its intravenous injection are illustrated diagrammatically in Figure 33. In the presence of a major iodide organification defect, most trapped ^{131}I remains in the gland as unorganified ^{131}I -iodide over a period of several hours. Perchlorate administration one or two hours later will discharge a large, easily quantitated fraction of total thyroid radioactivity. When the iodide organification defect is minor, however, thyroid content of unorganified

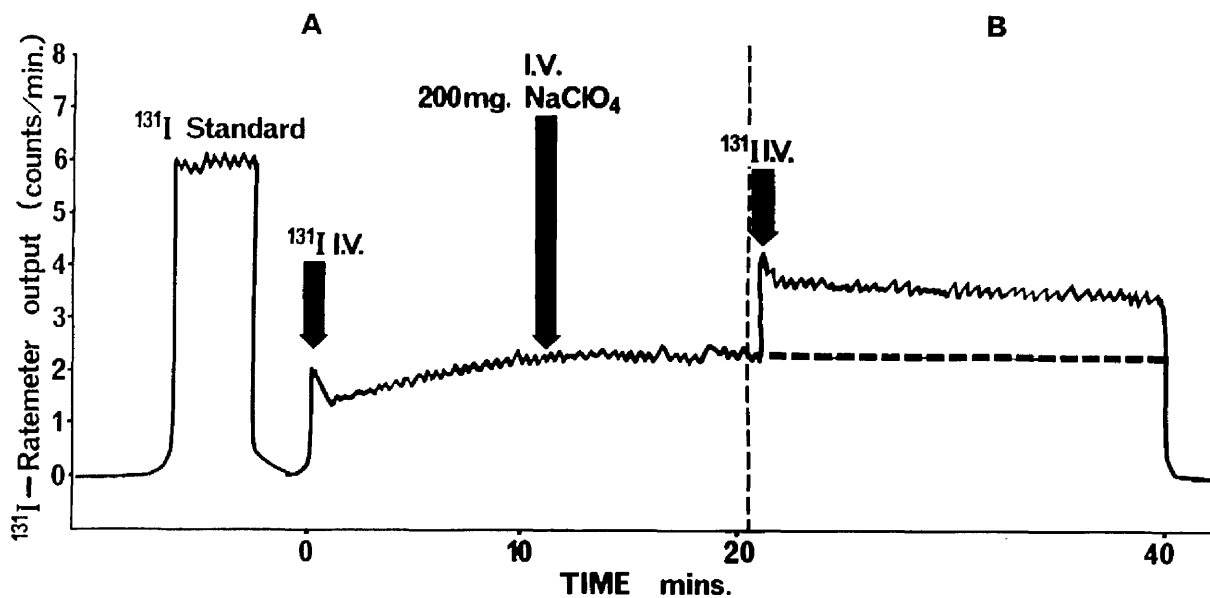


Figure 34: Trace record of neck radioactivity during the IV perchlorate test in a normal subject is shown in section A following the ^{131}I standard recording. Since iodide organification was normal, no discharge of ^{131}I was seen after IV perchlorate. Quantitation of ^{131}I ETA is illustrated in section B following IV injection of a second 25 μCi dose of ^{131}I .

^{131}I -iodide is high only in the first few minutes after its administration.

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^{131}I -iodide

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Figure 34. After

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was calculated as the

difference between total neck radioactivities at 10 and 20 minutes.

To determine whether the observed perchlorate discharge from 10 to 20 minutes was primarily due to discharge of thyroidal ^{131}I or to reduction in ETA which occurs with time (Chapter 5), the fall in contribution of ETA to total neck counts was measured between 10 and 20 minutes after ^{131}I administration by injecting a further 25 uCi ^{131}I intravenously at the end of the perchlorate test in 23 normal control patients. The procedure is shown in part (B) of Figure 34. It was assumed that total neck radioactivity from the initial dose of ^{131}I remained constant throughout the procedure and this level was used as a base-line. The increased neck radioactivity following the second ^{131}I dose was considered extrathyroidal since thyroid trap was blocked by perchlorate. Fall in this additional neck radioactivity was quantitated in percentage dose units between 10 and 20 minutes after the second ^{131}I injection and it was assumed that this measurement approximated the fall in contribution of ETA to total neck counts which would occur during the period of perchlorate discharge.

The intravenous perchlorate test was performed on 29 patients without thyroid disorder, 12 patients with untreated thyrotoxicosis, 16 patients with histologically proven Hashimoto's disease and 5 patients with Pendred's syndrome. No normal control subject had thyroid tissue palpable and each had a PB ^{127}I , 24 hour ^{131}I thyroid uptake and 48 hour PB ^{131}I result in the normal range. Each thyrotoxic patient, whose ages ranged from 30 - 50 years, had uncomplicated Graves' disease.



Figure 35: Anatomical relations of an enlarged rat thyroid. When performing hemithyroidectomy, care must be exercised to remove the small strap muscles which can be clearly seen overlying the lateral aspect of each thyroid lobe.

Fall in ^{131}I ETA from 10 to 20 minutes was measured in 23 normal control patients.

In subjects with thyrotoxicosis, Hashimoto's disease and Pendred's syndrome where ^{131}I ETA was not measured after the perchlorate test, the approximate thyroidal ^{131}I uptake at 10 minutes was calculated by subtracting 2% administered radioactivity, representing the 10 minute ETA, from total neck radioactivity at this time (Chapter 5).

Random errors of the technique were described in Chapter 5. Total random error of neck radioactivity measurement at 10 minutes was 20 - 25%.

(b) Rat In Vivo Study

Male Sprague-Dawley rats weighing 100 - 200 g were fed a normal diet. Following anaesthesia with Avertin - 1.5 ml of a 2.5% solution intraperitoneally - the (R) femoral vein was exposed by dissection.

Perchlorate Group

10 μCi ^{131}I in 0.2 ml saline was injected intravenously in each rat at time 0. The thyroid gland was exposed 2 minutes before perchlorate injection (Figure 35). 10 mg of sodium perchlorate in 0.1 ml was injected intravenously at either 4, 8 or 20 minutes after ^{131}I in groups of 9 rats. Immediately after perchlorate injection, the (L) thyroid lobe was rapidly dissected from trachea, a procedure taking approximately 30 seconds. The excised lobe was weighed on a torsion balance then placed in a sample tube containing 2 ml sodium hydroxide in preparation for counting. Digestion of the thyroid by alkali allowed more accurate counting of the tissue radioactivity.

The (R) thyroid lobe in each rat was removed 6 minutes after

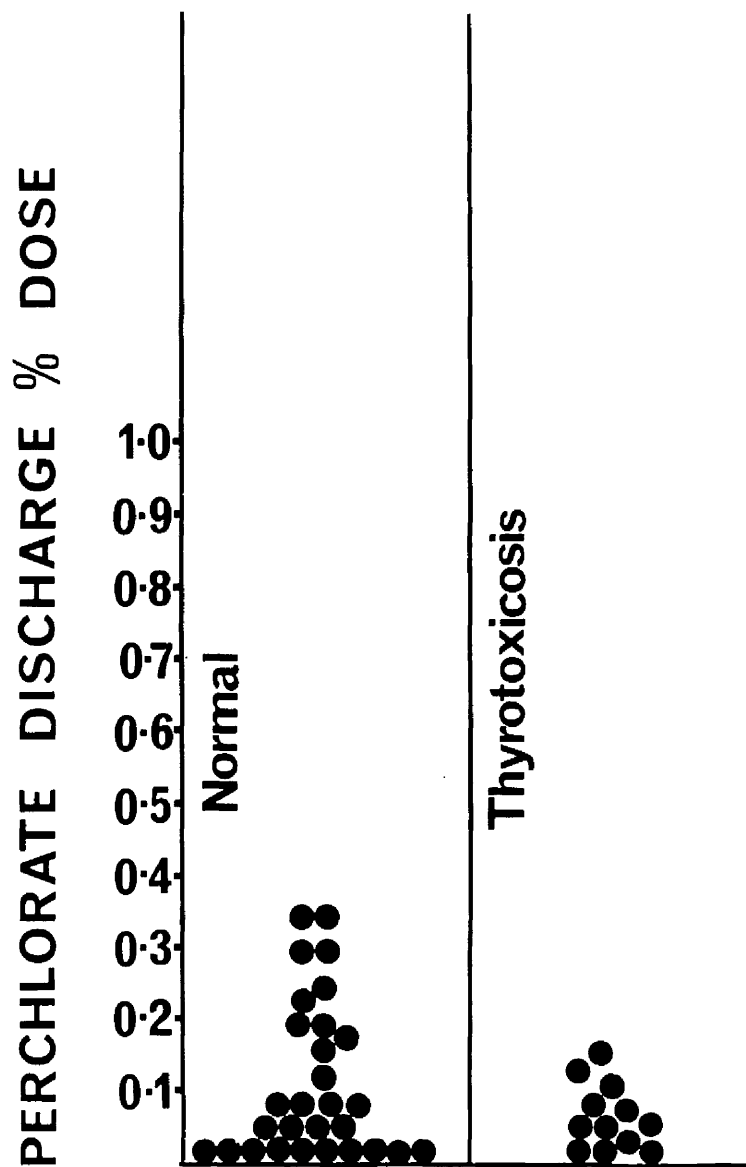


Figure 36: Twenty minute perchlorate test results in normal patients (29) and in untreated thyrotoxic patients (12).

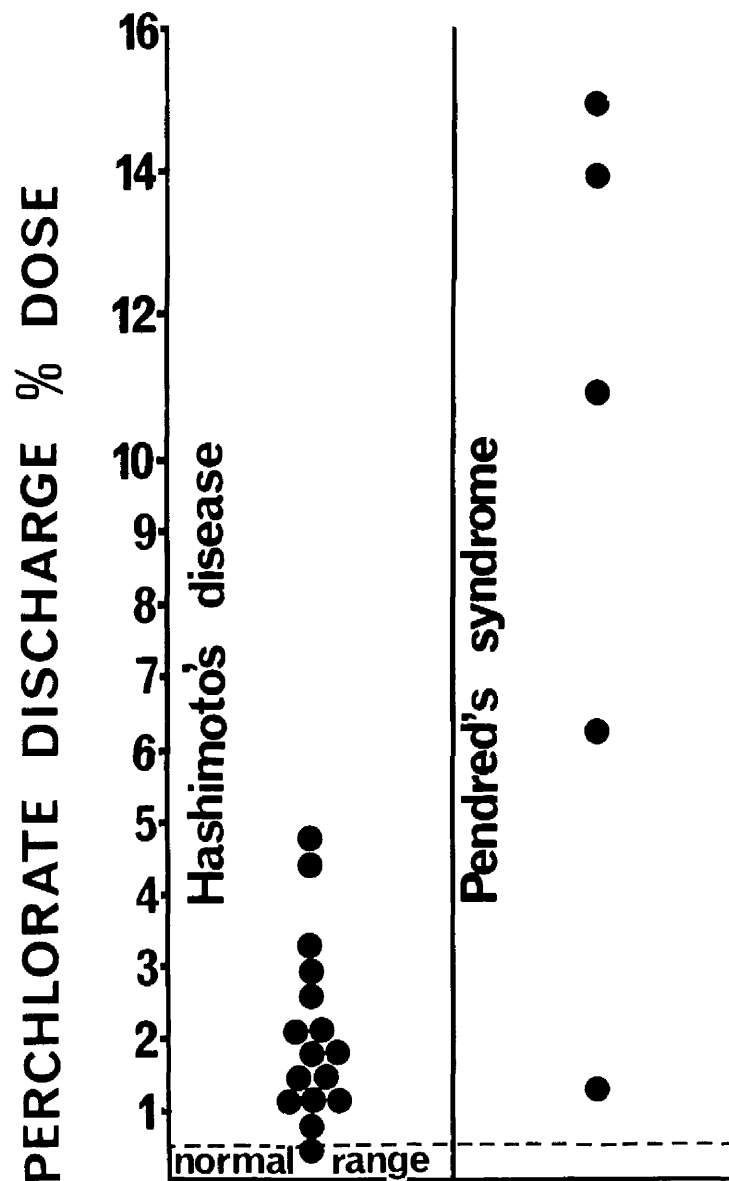


Figure 37: Twenty minute perchlorate test results in patients with histologically proven Hashimoto's disease (16) and in patients with Pendred's syndrome (5).

perchlorate injection since a preliminary study had indicated that discharge of thyroidal ^{131}I was completed within this time. This lobe was weighed and prepared for counting as before.

Control Group

Following intravenous ^{131}I as before in each rat at time 0, both thyroid lobes were removed simultaneously at either 4, 8 or 20 minutes after ^{131}I in groups of 4 rats. Each separate lobe was weighed and individually prepared for counting. In a further 8 rats, the (L) thyroid lobe was removed 4 minutes after ^{131}I injection and (R) lobe 6 minutes later without perchlorate administration. Lobes were individually weighed and prepared for counting as before.

Samples were counted 24 hours after the experiment in a well-type scintillation counter under standard conditions.

RESULTS

Human Study

Figure 36 In the 29 control patients, absolute thyroid radioactivity at 10 minutes was 3.1 ± 2.0 per cent administered dose (mean \pm 1SD) and perchlorate discharge between 10 and 20 minutes was 0.11 ± 0.13 per cent dose (mean \pm 1SD). In the 12 patients with thyrotoxicosis, approximate thyroid radioactivity at 10 minutes was 19.9 ± 10.5 per cent dose and perchlorate discharge was 0.05 ± 0.06 per cent dose.

Figure 37 In the 16 patients with Hashimoto's disease, approximate thyroid radioactivity at 10 minutes was 6.1 ± 2.8 per cent dose and perchlorate discharge between 10 and 20 minutes was 2.1 ± 1.2 per cent dose. In the 5 patients with Pendred's syndrome, approximate thyroid radioactivity at 10 minutes was 19.4 ± 7.0 per cent dose and perchlorate

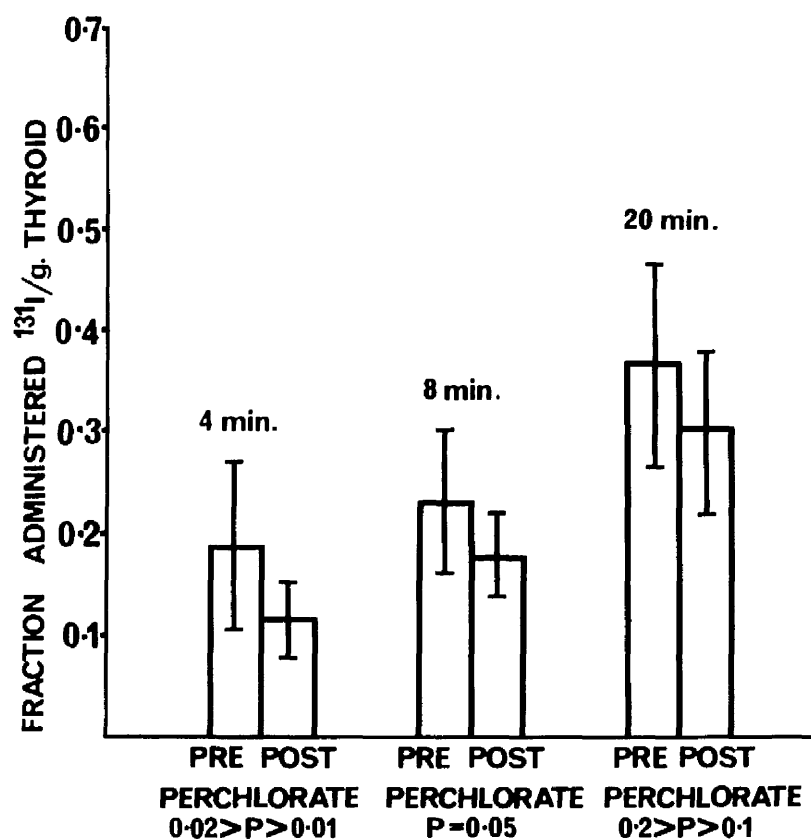


Figure 38: Results from the adapted perchlorate test procedure in normal non-goitrous rats. Thyroid concentration of ¹³¹I is shown before and 6 minutes after IV perchlorate injected at 4, 8 and 20 minutes after IV ¹³¹I.

discharge was 9.5 ± 5.7 per cent dose.

Fall in ETA of ^{131}I between 10 and 20 minutes after radionuclide administration in 23 control patients was 0.12 ± 0.25 per cent dose (mean \pm 1SD).

Animal Study

Effect of IV Perchlorate on Thyroidal ^{131}I Content Results are summarised in Figure 38. IV perchlorate 4 minutes after IV ^{131}I reduced mean thyroid uptake of ^{131}I by 38% from 0.186 ± 0.08 to 0.115 ± 0.037 fraction dose/g (mean \pm 1SD). This result was highly significant ($0.02 > P > 0.01$) using the paired 't' test. When perchlorate was injected at 8 minutes, mean thyroid uptake was reduced by 23% from 0.23 ± 0.07 to 0.176 ± 0.04 fraction dose/g. This result was significant ($P = 0.05$). With perchlorate injection at 20 minutes, mean thyroid uptake was reduced from 0.364 ± 0.10 to 0.30 ± 0.08 fraction dose/g. This result was not statistically significant ($0.2 > P > 0.1$) but was in keeping with the previous findings.

Control Studies

When both thyroid lobes were removed simultaneously in each of 4 rats at 4, 8 and 20 minutes after ^{131}I injection, there was no difference between the mean uptake of (L), 0.238 ± 0.072 and (R) lobe, 0.25 ± 0.05 fraction dose/g (mean \pm 1SD). In addition, when (L) lobe was removed 4 minutes after IV ^{131}I , the mean uptake, 0.142 ± 0.042 fraction dose/g (mean \pm 1SD), was significantly lower than the (R) lobe uptake at 10 minutes, 0.183 ± 0.061 fraction dose/g ($0.05 > p > 0.025$), if no perchlorate was given, indicating that

partial thyroidectomy did not affect function of the remaining lobe.

These control results confirm the validity of the procedure.

DISCUSSION

In the last 20 years, there have been conflicting views on whether iodide transport or organification provide the rate-limiting factor for hormone synthesis in human thyroid. Despite elegant in vivo studies by Ingbar and Frienkel (1958), De Groot and Davis (1961) and Nagataki and Ingbar (1963) which confirmed that rat thyroid accumulated ^{131}I in excess of organification, there have been no directly comparable studies to indicate that iodide organification is rate-limiting in humans. Using a perchlorate discharge test conceptually similar to that for humans, this in vivo animal study has confirmed the earlier observations, firstly that unorganified (dischargeable) thyroidal ^{131}I is present in rat thyroid, and secondly, that the amount present decreases with time probably in parallel with a falling plasma ^{131}I concentration. If these findings in rats are extrapolated to man, as advocated by Ingbar and Frienkel (1958), the human subjects with normal iodide organification should have discharged 20 - 25% of thyroidal ^{131}I content at 10 minutes (i.e. a mean of 0.75% dose in normal subjects and a mean of 5% dose in thyrotoxic subjects). Since no such discharge was demonstrated, the overall data clearly suggests that iodide organification is less efficient in rats than humans and indeed, that organification of transported ^{131}I is virtually instantaneous in humans with a K_{TB} of approximately unity, a view previously championed by Berson and Yalow (1955) and Vanderlaan

(1957). One can only presume that unorganified thyroidal iodide-127 detected in human thyroid by chemical analysis (Ingbar, 1955) forms part of the functionally distinct second iodide pool (Halmi and Pitt Rivers, 1962) or is an artefact due to in vitro deiodination.

Though of proven value in detection of major defects of iodide organification (Fraser et al, 1960), the conventional oral perchlorate discharge test is insensitive when the defect is minor. There are probably two main reasons for this limitation of the standard test.

Firstly, since thyroid content of ^{131}I must be quantitated for up to 40 minutes after perchlorate administration, each measurement of total neck uptake of ^{131}I requires individual correction for the contribution to it of ETA. This correction for ETA has usually been made indirectly (Fraser et al, 1960) and is not precise. Secondly, perchlorate is administered one or two hours after oral ^{131}I (Fraser et al, 1960; Floyd et al, 1960; Baschieri et al, 1963) at a time when thyroidal ^{131}I -iodide content is falling in parallel with the plasma ^{131}I level. Stewart and Murray (1966) recognised the importance of early perchlorate administration though delayed this till one hour after oral ^{131}I . Workers recently demonstrated that the clinical value of a conventional oral test for detection of Hashimoto's disease is enhanced by combining the oral dose of ^{131}I with carrier iodide (Takeuchi et al, 1970). It is problematical whether this expedient unmasks a true iodide organification defect or merely indicates undue susceptibility of iodide organification in Hashimoto's disease to an increase in trapped iodide.

Results of the intravenous perchlorate test in normal and untreated thyrotoxic patients indicate that fall in total neck radioactivity (thyroid + ETA) immediately following perchlorate is related primarily to fall in ETA of ^{131}I . This is suggestive but not conclusive evidence for absence of unorganified ^{131}I -iodide in glands with normal iodide organification. In contrast, patients with Hashimoto's disease all demonstrated a fall in neck radioactivity of more than 0.5 per cent dose following perchlorate. Clearly this indicates a decrease in thyroidal ^{131}I in addition to a fall in ^{131}I ETA and confirms that an iodide organification defect is a feature of Hashimoto's disease. It also suggests that the intravenous test is more sensitive than the oral test since Nilson and Berne (1964), Buchanan et al (1965) and Volpe et al (1965) reported that the oral perchlorate test was positive in only 30 - 50 per cent of patients with Hashimoto's disease. A detailed comparison between tests will be required to confirm this. As expected, all patients with Pendred's syndrome discharged a large percentage of administered dose. It is surprising, however, that no thyrotoxic patients demonstrated a positive test especially as 5 had positive anti-thyroid antibodies which presumably indicated the presence of thyroiditis.

As perchlorate discharge in the human control group was 0.11 ± 0.13 per cent dose (mean \pm 1SD), a discharge of $0.11 + 3\text{SD}$ has been accepted as the upper limit of normality, i.e. a discharge of 0.5 per cent dose. Discharge data has been presented as percentage of administered dose rather than percentage of thyroid uptake for two

reasons. Firstly, acknowledging random errors in the technique, false positives are less likely with this approach since the error associated with ETA measurement has been eliminated. Secondly, had the results been expressed as percentage of thyroid uptake discharged, quantitation of ^{131}I ETA with its inherent error would be necessary. This ETA measurement would require administration of an additional 25 uCi ^{131}I and the complete test would take 20 minutes longer to perform.

In conclusion this simple technique appears to allow detection of both major and minor iodide organification defects and should be of practical value in a busy Department of Nuclear Medicine. In subsequent chapters, the author reports findings from the use of this test in a variety of thyroid disorders.

CHAPTER 11 - SUMMARY

In a new approach to in vivo detection of defective iodide organification in thyroid, sodium perchlorate has been injected intravenously, 10 minutes after intravenous ^{131}I -iodide, to allow rapid performance of a perchlorate discharge test. Application of this test to normal and thyrotoxic patients has indicated that unorganified iodide cannot be detected in thyroid glands where iodide organification is normal. In contrast, detection of moderate amounts of unorganified iodide in normal rat thyroid by a similar discharge technique indicates a basic difference in thyroidal iodide metabolism between the species. The results imply that whereas iodide transport is the rate-limiting factor for normal hormone synthesis in humans, iodide organification is rate-limiting in rats. Where thyroidal iodide organification is defective in humans as a result of disease, the test has been shown to be a simple and efficient method of detecting the abnormality.

CHAPTER 12

THE ORGANIFICATION RATE OF THYROIDAL RADIOACTIVE IODIDE- ^{131}I : MEASUREMENT IN VIVO

In this chapter, a method is developed for in vivo measurement of the conversion rate of thyroidal radioactive iodide- ^{131}I to organically bound iodine- ^{131}I .

INTRODUCTION

In Chapter 11, a simple technique for detection of inorganic thyroidal radioactive iodide- ^{131}I (^{131}I) was presented. To enhance test sensitivity the results of this perchlorate test were expressed as per cent administered ^{131}I discharged from thyroid. Since the fraction of total thyroid ^{131}I uptake which discharges is not measured, however, the test is purely qualitative. Quantitation of iodide organification requires measurement of the thyroidal organification rate (K_{TB}).

Workers have measured K_{TB} in vivo using complicated analytic techniques (Berson and Yalow, 1955; Ingbar, 1955; Robertson et al, 1971) but results have been conflicting. The author has concluded from two pieces of evidence documented in this thesis that there is little, if any, inorganic iodide in thyroid glands of normal and untreated thyrotoxic patients. Firstly, no ^{131}I was dischargeable from thyroid in normal and thyrotoxic patients using the perchlorate test although a similarly conceived technique provided evidence for inorganic ^{131}I in normal rat thyroid (Chapter 11). Secondly, the

undoubted equivalence of thyroid net and unidirectional ^{131}I clearances (Chapter 8) make the presence of an unorganified iodide pool in thyroid, extremely unlikely. Since this argues that a defect of iodide organification is always associated with the presence of inorganic thyroid iodide, K_{TB} was measured only in those patients who demonstrated a perchlorate discharge of thyroid ^{131}I and it has been assumed that K_{TB} is near unity in glands where iodide organification is normal.

The proposed analysis is more simple than in any previous work and requires only the data from an intravenous perchlorate discharge test.

METHOD

The method is based on the following assumptions regarding thyroid metabolism of iodide.

(a) Perchlorate completely blocks iodide transport

This assumption is based on the following observations.

1. When 50 - 250 mg of sodium perchlorate is injected intravenously, and one minute later, ^{131}I is injected by the same route, measurement of neck radioactivity with directional counting reveals a gradual reduction in radioactivity over 20 minutes.
2. An identical pattern of falling neck radioactivity is seen in athyreotic patients (Gray, 1973) when ^{131}I is injected without prior perchlorate.

- (b) Where defective iodide organification is present in human thyroid, a three compartment model (Chapter 4) accurately portrays thyroidal ^{131}I kinetics

The validity of this assumption is based on the pioneer work of Wollman and Reed (1962a) in rats.

- (c) Neck extrathyroidal radioactivity (ETA) at 10 minutes is 2% administered dose

For validity see Chapter 5.

- (d) Perchlorate acutely reduces K_{TB} to zero in patients with defective iodide organification

Evidence in support of this can be marshalled as follows.

When perchlorate blocks thyroid iodide transport, inorganic iodide in thyroid may theoretically diffuse to plasma via K'_{TP} and/or be organified via K_{TB} . Without making assumptions on the effect of perchlorate on K_{TB} , it can be proven mathematically (Appendix 3) and demonstrated with analogue computation (Gray, 1973) that the observed exit-rate constant for iodide, calculated from the observed perchlorate discharge curve, is the sum of the actual exit-rate constant and the organification rate constant.

$$K'_{\text{TP}} (\text{observed}) = K'_{\text{TP}} (\text{actual}) + K_{\text{TB}}$$

Since thyroid $^{99\text{m}}\text{Tc}$ remains unorganified, the observed K'_{TP} for $^{99\text{m}}\text{Tc}$ is always the actual K'_{TP} and this measurement can be regarded as a base-line diffusion rate for any particular patient. When ^{131}I K'_{TP} is measured, it is clear that the observed value will be greater than the actual value if K_{TB} is

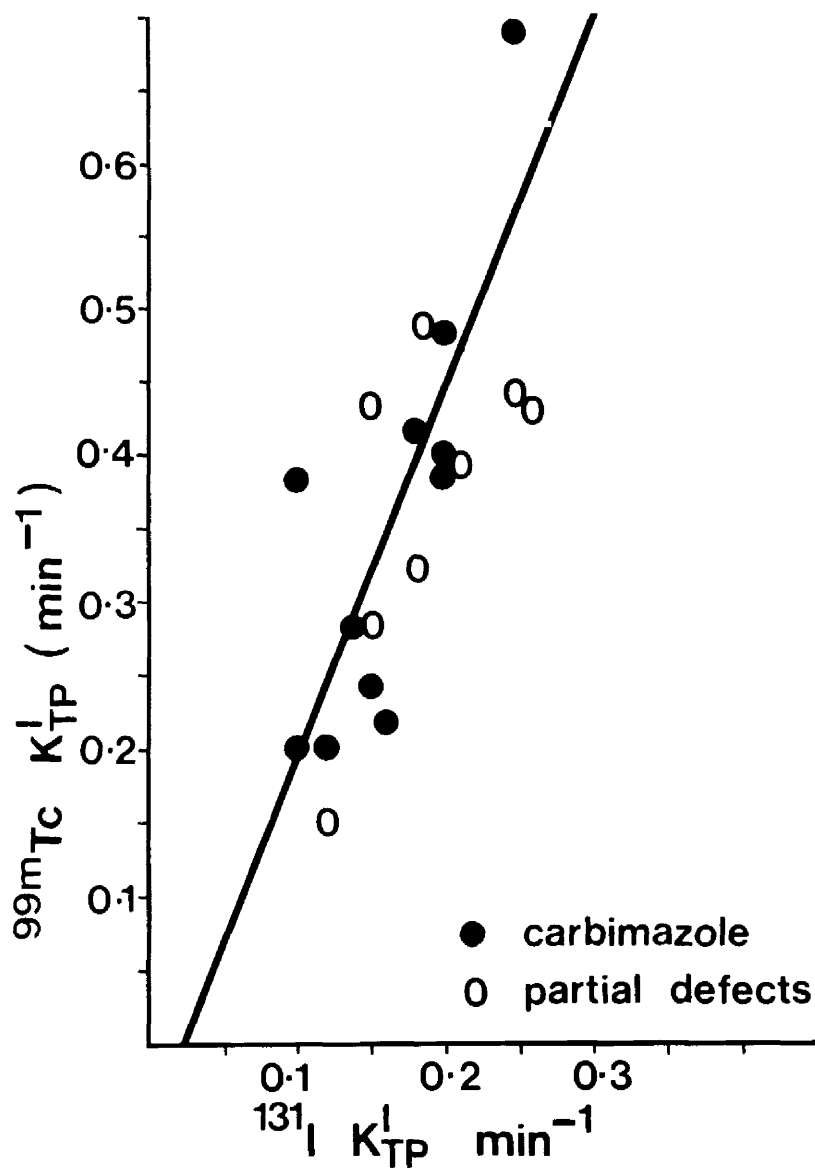


Figure 39: This figure shows the correlation between K'_{TP} of ^{99m}Tc and ^{131}I measured in 8 patients with partial defects of iodide organification (open circles), superimposed on the same correlation from thyrotoxic patients on carbimazole (closed circles - see Figure 20). Statistical analysis confirms that both sets of data could be drawn from the same population.

the
unaffected by perchlorate and that/total discharge of thyroid
iodide will not represent the original amount of thyroïdal
iodide present when perchlorate was given in this situation.
The present studies however have revealed

- (a) that in thyrotoxic patients currently on carbimazole therapy
where K_{TB} may be assumed zero, K'_{TP} for ^{131}I and ^{99m}Tc showed
direct proportionality with the ^{99m}Tc value approximately equal
to $2\frac{1}{2} \times ^{131}I$ value (Figure 20, Chapter 8). The gradient mean
 \pm LSD was 2.56 ± 1.25 .
- (b) Further, that in patients with partial defects of iodide
organification where $1 > K_{TB} > 0$, K'_{TP} of ^{99m}Tc was $1.5 \times ^{131}I$
value (Figure 39). The gradient mean \pm LSD was 1.5 ± 0.65 .

Since there is no statistical difference between gradients observed
in both groups of patients, the author submits that perchlorate
administration in patients with partial defects of iodide organification
considerably reduces the efficiency of an already compromised
organification mechanism. It is therefore likely that ^{131}I discharged
from thyroid by perchlorate approximates to the total amount of inorganic
 ^{131}I present in ^{the} gland when perchlorate is administered.

This hypothesis is not difficult to reconcile with the observation
of De Groot and Buhler (1971) that prolonged perchlorate administration
did not interfere appreciably with re-organification of second-pool
iodide. It is probable that perchlorate induces significant
inhibition of iodide organification only when K_{TB} is already compromised
by disease. A modest reduction in normal K_{TB} would hardly prevent the

acknowledged slower organification of second-pool iodide documented by De Groot. Indeed Greer et al (1966) have produced convincing evidence that perchlorate can inhibit iodide organification in normal rat thyroid where, as the author has shown (Chapter 11), K_{TB} is < 1 . It would appear likely that a defective iodide organification mechanism in humans is more sensitive than normal to perchlorate thus providing a less than tenuous explanation for the author's findings.

KINETIC ANALYSIS

From the three-compartment model of thyroid iodide metabolism where

B = organified thyroidal ^{131}I (fract. dose)

F = unorganified thyroidal ^{131}I (fract. dose)

K_{TB} = ^{131}I organification rate constant (min^{-1})

$$B(t_1) = K_{TB} \int_0^{t_1} F$$

Reorganising this equation and differentiating,

$$\frac{dB/dt}{F} (t_1) = K_{TB}$$

To obtain K_{TB} from an intravenous perchlorate test therefore, both rate of change of organified thyroidal ^{131}I (B) at 10 minutes and quantity of unorganified thyroidal ^{131}I (F) at this time are required. F can be measured from the drop in thyroid radioactivity after perchlorate. Assuming ETA at 10 minutes, total thyroidal ^{131}I at 10 minutes can be measured and subtracting F , organified thyroidal ^{131}I (B) can be calculated. Solution to the final problem of calculating dB/dt at

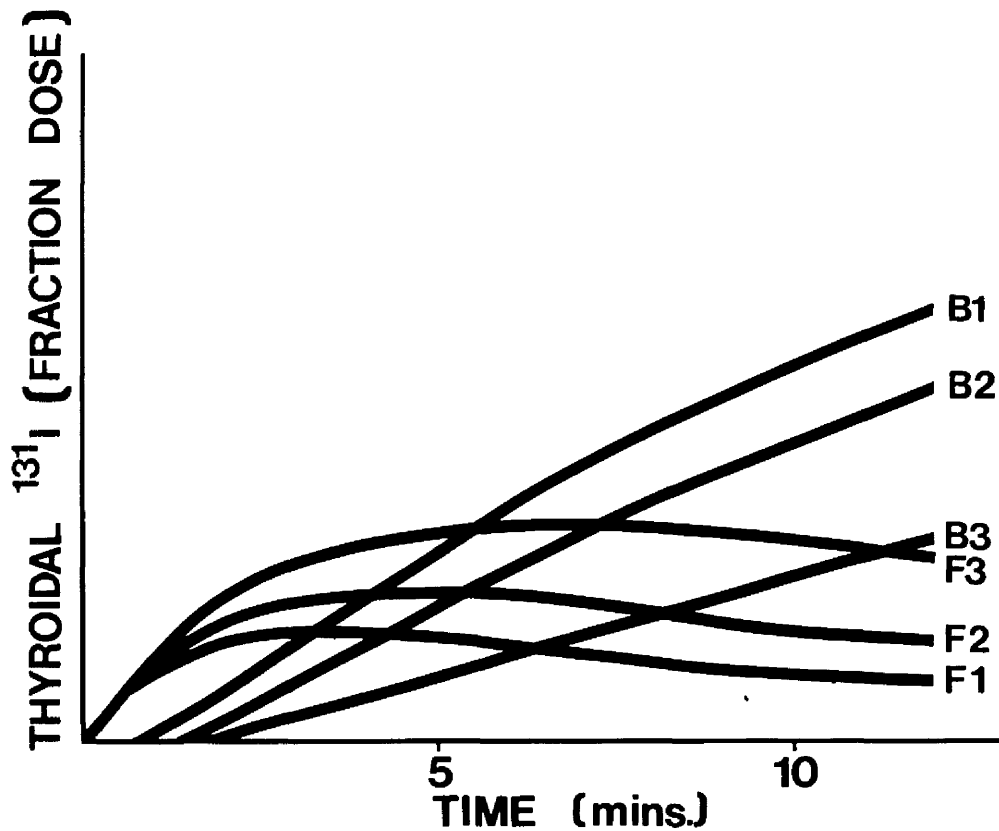


Figure 40: Examples of pairs of thyroidal ^{131}I uptake curves produced by the analogue computer representing organified (B) and inorganic ^{131}I (F) over a 10 minute period after IV ^{131}I injection. For preparation of the three pairs of curves displayed, unidirectional clearance (C) and exit-rate constant (K_{TF}) were held constant and the organification-rate constant (K_{TB}) was varied. K_{TB} was 0.40 for curves F_1/B_1 , 0.25 for F_2/B_2 and 0.10 for F_3/B_3 .

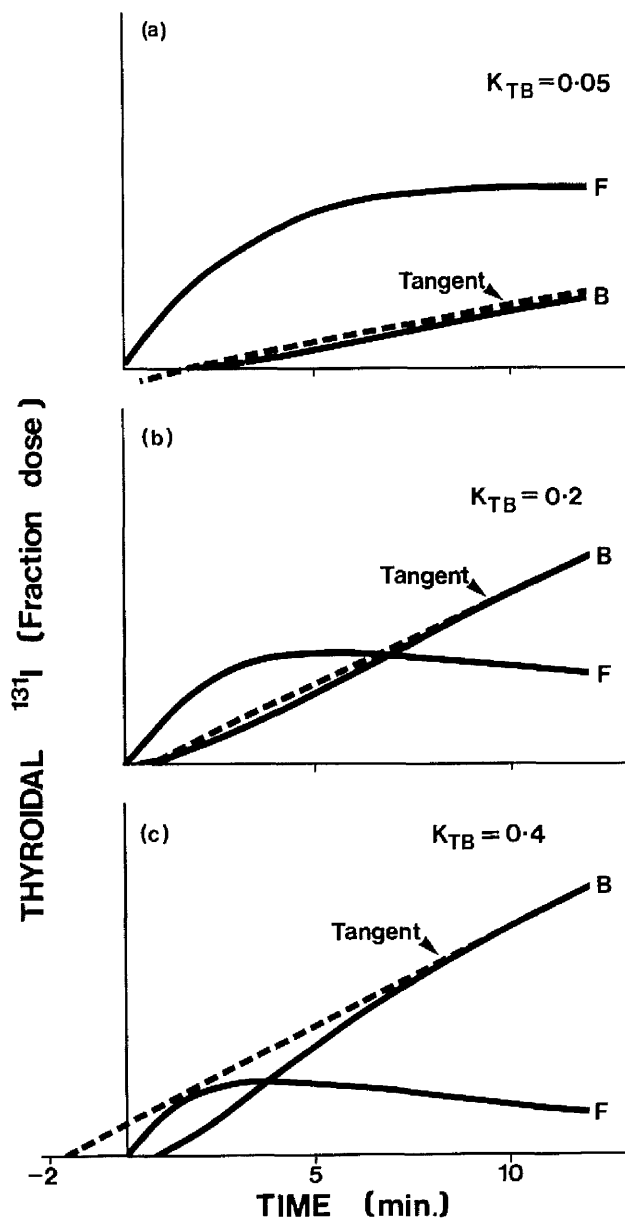


Figure 41: Pairs of thyroidal organified (B) and inorganic (F) ¹³¹I curves over 10 minutes post IV injection as portrayed by the analogue computer. When $F > B$ at 10 minutes (a), the tangent to B curve at 10 minutes intercepted time axis at approximately + 2 minutes. When $4F > B > F$ at 10 minutes (b), the tangent to B curve at 10 minutes intercepted time axis at approximately zero minutes. When $B > 4F$ and $K_{TB} < 0.40$, the tangent to B curve at 10 minutes intercepted time axis at approximately - 2 minutes.

10 minutes was indicated by studies with an analogue computer.

Calculation of dB/dt (10 min)

Simulation of a three-compartment thyroid model with analogue computation (Appendix 2), allowed production of pairs of thyroidal ^{131}I uptake curves, representing organified (B) and unorganified (F) ^{131}I content with time. In this study, various plasma ^{131}I decay curves were used and each of the three rate constants of the model (C , K_{TP} , K_{TB}) were varied. Examples are shown in Figure 40. The following were observed from curves produced.

1. A predictable effect of rise in K_{TB} was an increase in both rate of rise of B curve and rate of fall of F curve.
2. The B curve, integral of $F \times K_{TB}$, was virtually a straight line for $K_{TB} < 0.10$. With $0.10 < K_{TB} < 0.40$, the B curve was a straight line from the simulated 7th minute onwards.

dB/dt at 10 minutes is clearly obtained from measurement of the slope of the B curve at that time. It was found that

1. when $F > B$ at 10 minutes, the 10 minute tangent to B curve intercepted time axis at approximately 2 minutes (Figure 41a).
2. When $4F > B > F$ at 10 minutes, the tangent to B curve intercepted time axis at approximately zero minutes (Figure 41b).
3. When $B > 4F$ at 10 minutes, the 10 minute tangent to B curve intercepted time axis at approximately minus 2 minutes up to a K_{TB} of approximately 0.40 (Figure 41c).

Using this information, a reasonable approximation to dB/dt at 10 minutes could be obtained as follows:

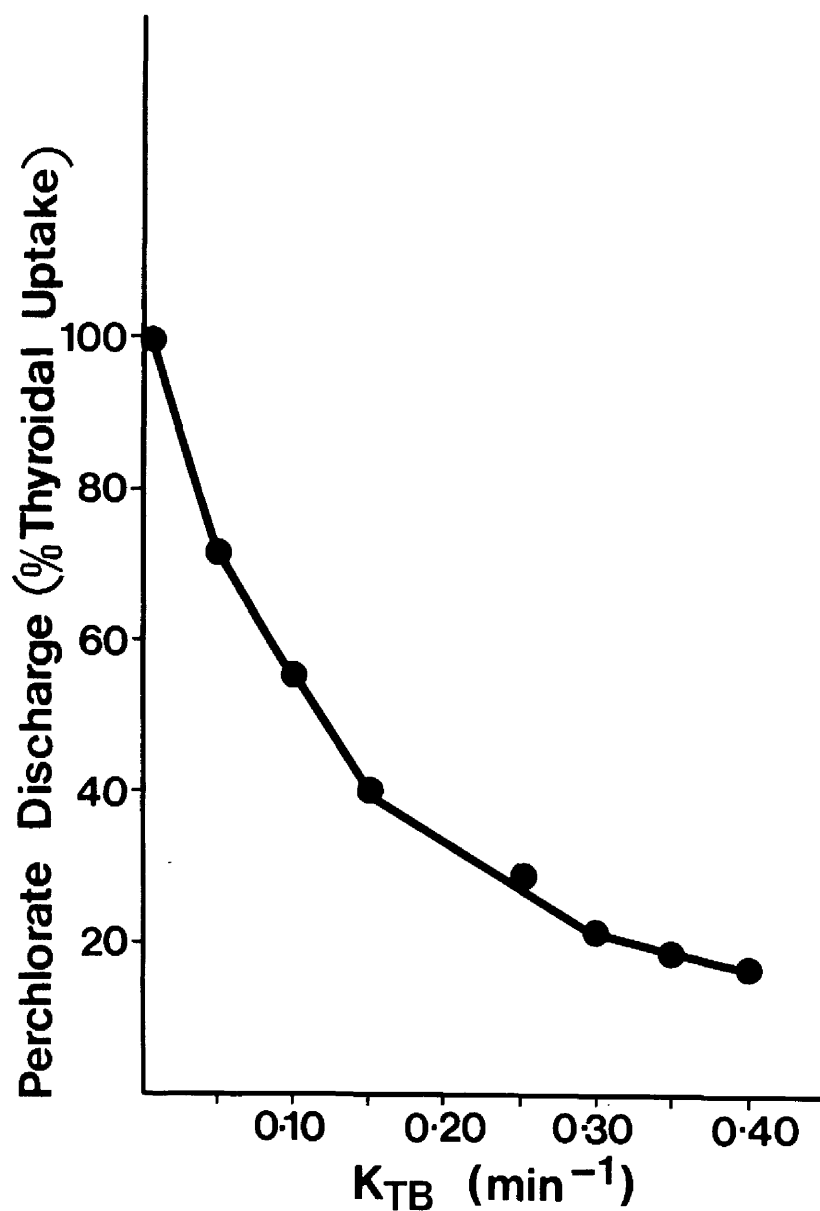


Figure 42: This graph shows the non-linear relationship between the organification-rate constant (K_{TB}) of thyroidal inorganic ^{131}I and the percentage of thyroid uptake discharged at 10 minutes by perchlorate. The graph was constructed using the equations developed in this thesis.

- (a) When $F > B$, (dB/dt) at 10 min = $\frac{B \text{ at (10 min)}}{8}$
- (b) When $4F > B > F$, (dB/dt) at 10 min = $\frac{B \text{ at (10 min)}}{10}$
- (c) When $B > 4F$, (dB/dt) at 10 min = $\frac{B \text{ at (10 min)}}{12}$ for $K_{TB} \leq 0.40$

With the above approximations and by studying many pairs of F and B curves produced on the analogue computer using varied plasma ^{131}I curves and variable C, K_{TP} and K_{TB} , the author found that:

- (i) Variation in plasma ^{131}I curve and constant C had no effect on dB/dt approximation, K_{TP} and K_{TB} being constant.
- (ii) Though large K_{TP} values > 0.15 were an increasing source of error in dB/dt approximation, K_{TP} variation in the physiological range 0.05 to 0.12 (Chapter 8) had little effect.
- (iii) Error of dB/dt estimation using the chord approximation with $0.05 \leq K_{TP} \leq 0.12$ was a maximum of 13% provided $K_{TB} \leq 0.40$. When K_{TB} increased beyond 0.40, the error became unacceptably large.

It appeared therefore, that using the intravenous perchlorate test with perchlorate injection at 10 minutes, one can predict that:

- (a) when $F > B$, $K_{TB} = \frac{B}{8F}$
- (b) when $4F > B > F$, $K_{TB} = \frac{B}{10F}$
- (c) when $B > 4F$ and $K_{TB} \leq 0.40$, $K_{TB} = \frac{B}{12F}$

Using these equations, the approximate relationship between K_{TB} and perchlorate discharge of unorganified thyroidal ^{131}I as a percentage of total thyroidal ^{131}I uptake, may be portrayed (Figure 42). This

TABLE 8

THE ^{131}I ORGANIFICATION RATE CONSTANT (K_{TB}) IN POPULATIONS OF PATIENTS
WITH SEPARATE THYROID DISEASES CHARACTERISED BY DEFECTIVE IODIDE ORGANIFICATION

<u>PATIENT GROUPS</u>	<u>THYROID K_{TB} (min^{-1})</u>	<u>SERUM $\text{PB } ^{127}\text{I}$ ($\mu\text{g}\%$)</u>
Hashimoto's disease (n = 15)	0.216 ± 0.096	4.6 ± 1.5
Pendred's syndrome (n = 3)	0.23 ± 0.12	8.4 ± 1.5
Thyrototoxicosis - post radiation therapy (n = 5)	0.21 ± 0.07	7.75 ± 3.2

Mean value and 70% confidence limits are shown

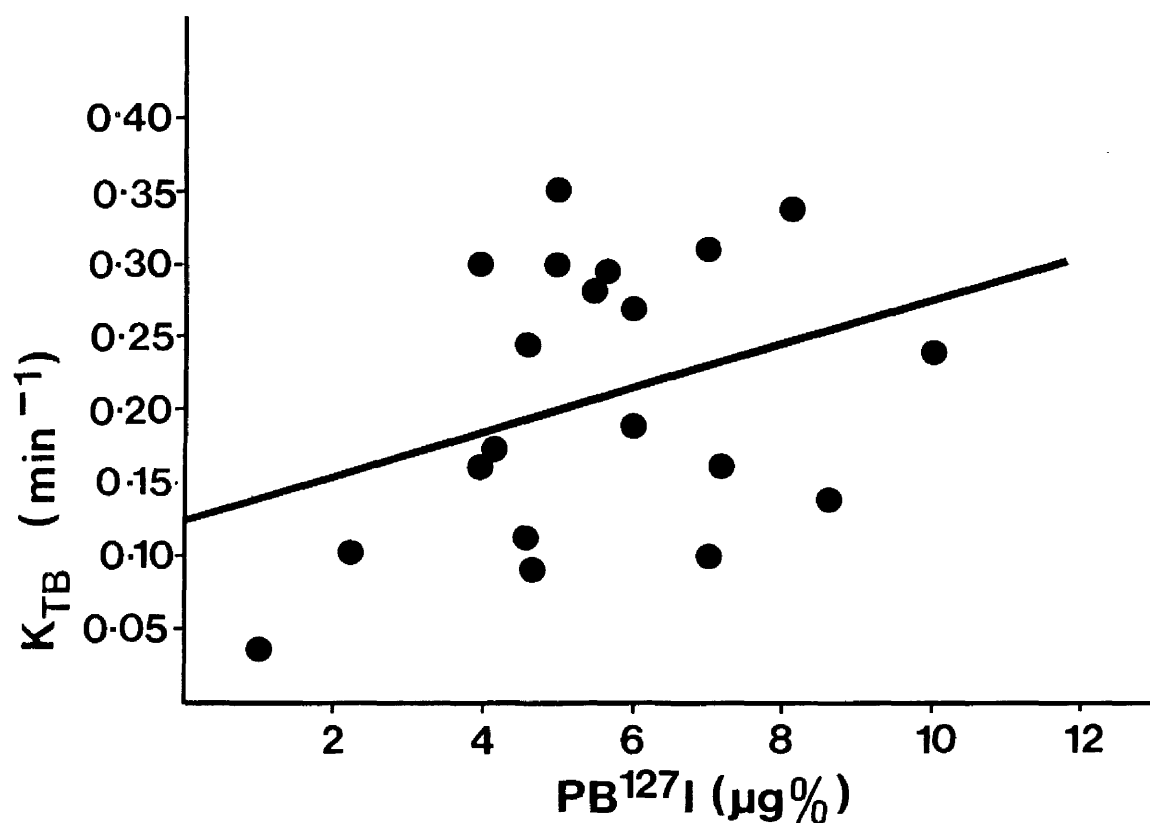


Figure 43: This figure shows the poor correlation ($r = 0.35$) obtained between thyroid status as measured by a PB^{127}I and the thyroïdal ^{131}I organification-rate constant (K_{TB}). Subjects studied were 15 patients with Hashimoto's disease, 5 patients following radioactive iodine treatment for thyrotoxicosis and 3 patients with Pendred's syndrome.

graph eliminates the necessity for separate calculation in each patient studied and requires only that the fraction of total thyroid uptake of ^{131}I which is discharged at 10 minutes is known.

PATIENTS STUDIED AND PROCEDURE

Of 23 patients studied, 15 had clinical evidence of Hashimoto's disease (goitre and strongly positive tests for serum anti-thyroid antibodies), 5 had received a therapy dose of radiiodine (^{131}I or ^{125}I) for thyrotoxicosis 4 - 8 months prior to test, and 3 had Pendred's syndrome. In each patient, an intravenous perchlorate test was performed (Chapter 11) though the test was terminated only when thyroidal ^{131}I discharge had ceased. Total thyroidal ^{131}I radioactivity at 10 minutes was calculated by subtracting 2% dose (approximate ETA) from total neck uptake at that time. Overall thyroid status was assessed by a PB^{127}I measurement. K_{TB} for each patient was obtained from Figure 42, using the percentage of total thyroidal uptake of ^{131}I discharged by perchlorate.

RESULTS

The results are summarised in Table 8 and portrayed graphically in Figure 43. A poor correlation ($r = 0.35$) between ^{131}I K_{TB} and PB^{127}I was seen in the group of patients studied.

DISCUSSION

The method presented for approximation of ^{131}I K_{TB} has the virtue of technical simplicity over other techniques (Berson and Yalow, 1955; Ingbar, 1955; Robertson et al, 1971). A poor correlation between

K_{TB} and ^{127}I indicates that K_{TB} measurement is of limited value as an index of total thyroid function. This is hardly surprising since a radioactive rate constant allows a meaningful comparison between subjects only when the stable pool (in this case, inorganic thyroid iodide) to which it pertains is either known or is of comparable size in different subjects. Clinical usefulness of K_{TB} measurement is clearly limited by variation in size of the thyroid inorganic iodide pool between patients with different thyroid pathologies and between different patients with the same thyroid pathology. Its measurement could theoretically be useful for sequential analysis in the same patient where the iodide pool might be expected to remain constant. This possibility has been explored in Chapter 14.

It is interesting that the relationship between fraction of thyroid uptake discharged and K_{TB} is non-linear (Figure 42). There would appear little merit therefore, in the time-honoured presentation of perchlorate test data as fraction of thyroid uptake discharged, since K_{TB} approximation requires knowledge of this graph. This fact, coupled with the unsatisfactory correlation of ^{131}I K_{TB} with thyroid status, argues for presentation of intravenous perchlorate test data as percentage administered dose discharged. Although the test will thereby remain purely qualitative, sensitivity is clearly enhanced by this approach.

In conclusion, measurement of ^{131}I K_{TB} has little place in clinical investigation and until it is possible to quantitate ^{127}I K_{TB} , an intravenous perchlorate test per se is the most appropriate procedure for assessment of thyroid iodide organification.

SUMMARY - CHAPTER 12

A simple theoretical analysis has been developed for quantitation of thyroidal iodide organification. Extensive use of analogue computation permitted formulation of basic equations which defined K_{TB} , organification rate of ^{131}I , in terms of the fraction of total thyroidal ^{131}I content at 10 minutes which is discharged by perchlorate.

The calculated ^{131}I K_{TB} correlated poorly with thyroid status (PB^{127}I), presumably because of inter-patient variation in size of the stable thyroid iodide pool. It was tentatively suggested that K_{TB} measurement could be useful for sequential analysis of iodide organification in individual patients.

CHAPTER 13

HASHIMOTO'S DISEASE: ITS DIFFERENTIATION FROM SIMPLE GOITRE USING THE INTRAVENOUS PERCHLORATE DISCHARGE TEST

In this chapter, the intravenous perchlorate discharge test is applied to the differential diagnosis of non-toxic goitre, and a role for this test in the definitive diagnosis of Hashimoto's disease is proposed.

INTRODUCTION

Differentiation of Hashimoto's disease from simple goitre on purely clinical grounds is often difficult when the patient is euthyroid. Routine thyroid function tests may also be equivocal in both conditions (Murray and McGirr, 1960; Buchanan et al, 1965; Shane et al, 1965) and although high titres of serum anti-thyroid antibodies are diagnostic of Hashimoto's disease (Roitt and Doniach, 1958), low titres are not, since these are found in 20 - 30% of patients with simple goitre (Doniach et al, 1960). Needle biopsy of thyroid appears to be the most definitive diagnostic technique (Beahrs et al, 1962; Heimann and Schnurer, 1964) but despite a negligible morbidity associated with its use, popularity for the procedure varies from centre to centre (Williams, 1968).

The intravenous perchlorate discharge test was used to study 112 patients who presented with non-toxic goitre. The author's aim was to determine whether the defective thyroid organification of iodide,

found in Hashimoto's disease (Chapter 11) could allow its accurate differentiation from simple goitre. The author's experience with the test, the results and their diagnostic utility are discussed.

MATERIALS AND METHOD

Patients Studied and Criteria Adopted

A total of 112 patients were studied. All attended the Thyroid Clinic at the Royal Infirmary, Glasgow with a non-toxic goitre larger than a clinically estimated 40 g. Patients with suspected carcinoma of thyroid were not included in the study.

An ultimate diagnosis of Hashimoto's disease was made in a total of 53 patients. This diagnosis was confirmed in 22 by histological examination after thyroidectomy (16) or needle biopsy of thyroid (6). In the remaining 31 patients, diagnosis of Hashimoto's disease was made because they were either hypothyroid with positive tests for serum anti-thyroid antibodies (26) or euthyroid with strongly positive tests for these antibodies (5).

An ultimate diagnosis of simple goitre was made in a total of 40 patients. This diagnosis was confirmed in 10 by histological examination after thyroidectomy. In the remaining 30 patients, diagnosis of simple goitre was made because they were euthyroid with negative tests for serum anti-thyroid antibodies and had a ^{131}I \leq 0.2% dose/litre at 48 hours.

Of 112 patients studied, 19 were not included in the results. These were:

1. Ten patients in whom data collection was incomplete.

2. Two euthyroid patients with negative antibody tests but a $\text{PB}^{131}\text{I} > 0.3\%$ dose.
3. Five euthyroid patients with low titres of anti-thyroid antibodies in whom a biopsy was not obtained.
4. Two patients with different diseases. One had amyloid goitre, the other Riedels thyroiditis.

Immunological Studies

Precipitating anti-thyroglobulin auto-antibodies were detected by the Ouchterlony plate technique (Anderson et al, 1962). Anti-thyroglobulin was detected by the tanned red cell haemagglutination test described by Fulthorpe et al (1961) using thyroglobulin-coated formolized tanned sheep red cells (Burroughs Wellcome).

Anti-microsomal auto-antibody was measured using an immunofluorescent technique (Holborow et al, 1959).

Patients were considered to have high titres of antibodies when the precipitin test was positive and/or CF test was strongly positive and low titres when TRC was $< 1/5$ to $1/250$ and/or CF test was weakly positive.

Studies of Iodine Metabolism

Measurement of 48 hour PB^{131}I was performed as described by Wayne et al (1964). Thyroid status was confirmed by either a PB^{127}I or total serum thyroxine measurement using the Thyopac-4 kit (Radiochemical Centre, Amersham). The intravenous perchlorate test was performed as previously described (Chapter 11).

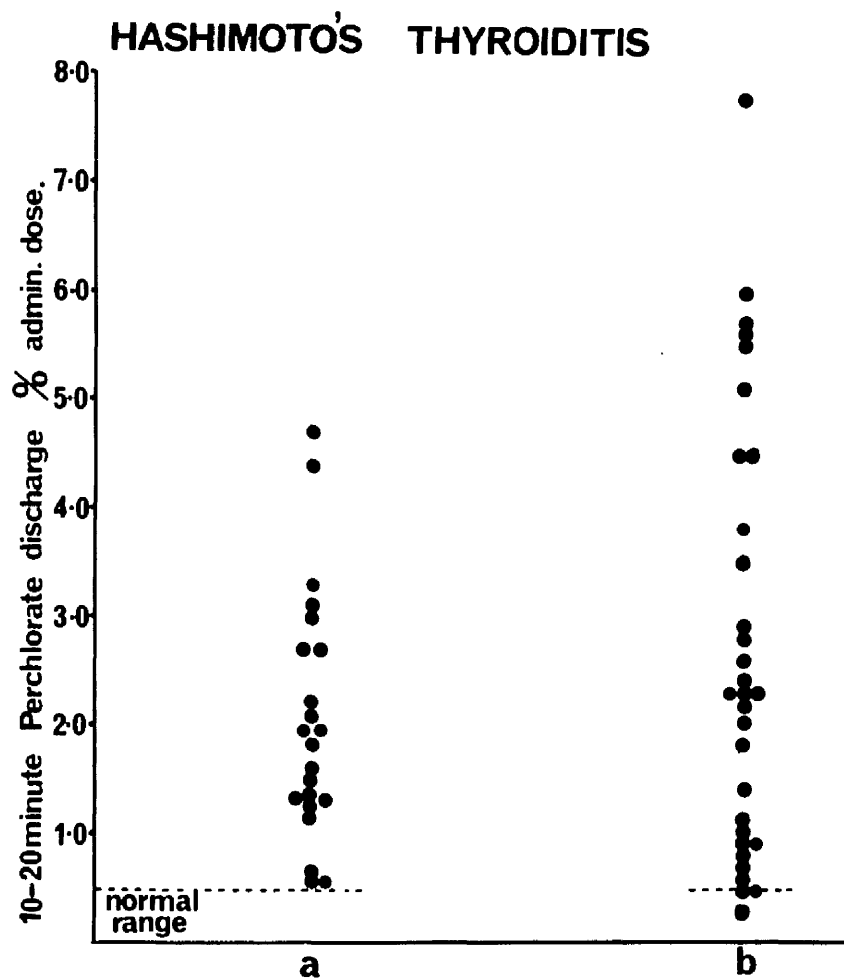


Figure 44: The results of the IV perchlorate discharge test in 53 patients with Hashimoto's disease. Group 'a' represents patients with a histological diagnosis (22) and group 'b', patients in whom the diagnosis was considered likely (31).

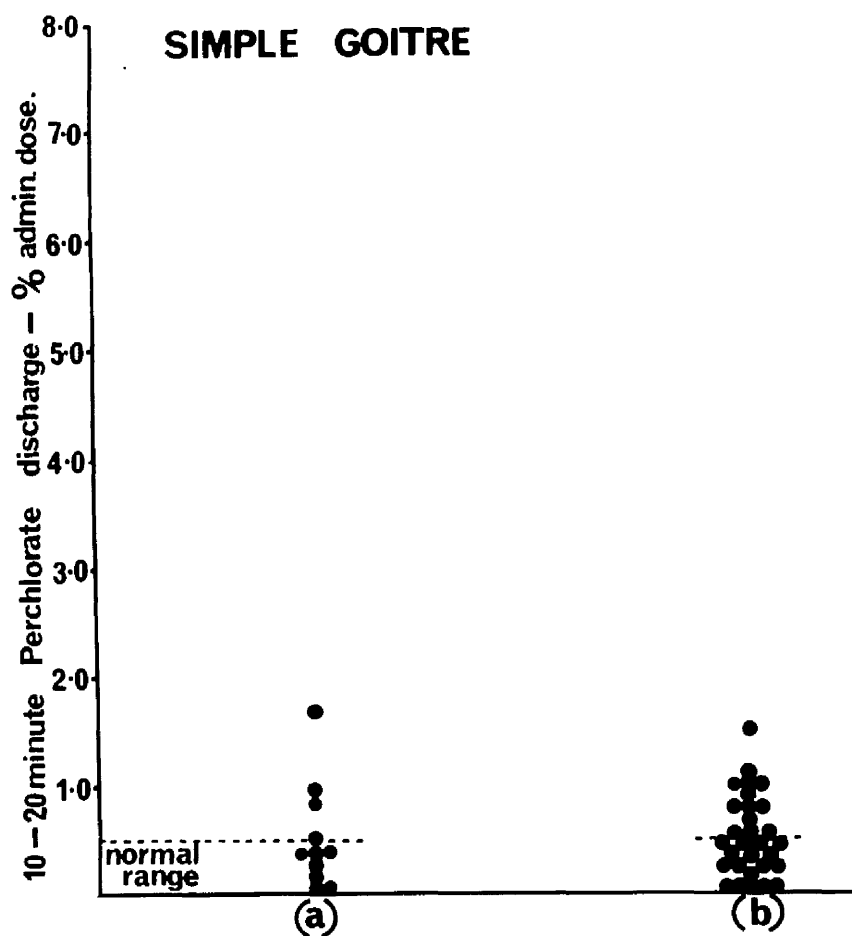


Figure 45: The results of the IV perchlorate discharge test in 40 patients with simple goitre. Group 'a' represents patients with a histological diagnosis (10) and group 'b', patients in whom the diagnosis was considered likely (30).

TABLE 2

RESULTS OF THE INTRAVENOUS PERCHLORATE TEST IN PATIENTS WITH

HASHIMOTO'S DISEASE AND SIMPLE GOITRE

<u>Criterion for Positive Test</u>	<u>Diagnosis of Hashimoto's Disease</u>		<u>Diagnosis of Simple Goitre</u>	
	<u>Histological</u>	<u>Probable</u>	<u>Probable</u>	<u>Histological</u>
Discharge greater than 0.5% dose	100% (22)	90% (28)	50% (15)	40% (4)
Discharge greater than 1.0% dose	87% (19)	74% (23)	13% (4)	10% (1)

Number of patients in parenthesis

TABLE 10

SERIES OF PATIENTS WITH NON-TOXIC GOITRE WHO PRESENTED A

PROBLEM IN CLINICAL DIAGNOSIS

<u>PATIENT</u>	<u>HISTOLOGICAL DIAGNOSIS</u>	<u>ANTIBODY TITRE</u>	<u>THYROID STATUS</u>	<u>PERCHLORATE DISCHARGE % DOSE</u>	<u>DIAGNOSIS FROM IV TEST</u>
1	Simple goitre	+	Euthyroid	0.23	Correct
2	Simple goitre	+	Euthyroid	0.00	Correct
3	Simple goitre	+	Euthyroid	0.00	Correct
4	Hashimoto's disease	+	Euthyroid	2.7	Correct
5	Hashimoto's disease	+	Euthyroid	1.3	Correct
6	Hashimoto's disease	neg	Euthyroid	1.8	Correct
7	Simple goitre	neg	Euthyroid	1.66	Incorrect

The antibody titre of + indicates a TRC titre of less than 1/5 to 1/250 and/or a weakly positive CF titre

RESULTS

Results of the perchlorate test in the 53 patients with Hashimoto's disease are shown in Figure 44. Twenty-two patients in whom histological proof of diagnosis was obtained discharged $2.04 \pm 1.14\%$ dose (mean \pm LSD) while 31 patients in whom the diagnosis appeared to be Hashimoto's disease with a high degree of probability discharged $2.78 \pm 1.99\%$ dose (mean \pm LSD).

Results of the perchlorate test in the 40 patients with simple goitre are shown in Figure 45. Ten patients in whom histological proof of diagnosis was obtained discharged $0.51 \pm 0.51\%$ dose (mean \pm LSD) while 30 patients judged likely to have a simple goitre discharged $0.51 \pm 0.35\%$ dose (mean \pm LSD). The difference between Hashimoto and simple goitre groups as a whole, was highly significant ($p < .001$).

The degree of discrimination obtained between Hashimoto's disease and simple goitre using the perchlorate test alone is shown in Table 9. With ^{the} standard criterion for a positive test (discharge $> 0.5\%$ dose) an average of 95% of patients with Hashimoto's disease and 45% with simple goitre showed a positive test. When the criterion for test positivity was revised to $> 1.0\%$ dose discharge, an average of 80% of patients with Hashimoto's disease but only 11.5% with simple goitre showed a positive test.

Data on 5 euthyroid patients with low titres of serum anti-thyroid antibodies where the clinical diagnosis was obscure is shown in Table 10. The perchlorate test correctly categorised each patient before thyroidectomy. Also documented are 2 euthyroid patients with negative

tests for serum anti-thyroid antibodies. The perchlorate test led to an incorrect diagnosis in 1 of those patients.

DISCUSSION

The incidence of positive oral perchlorate discharge tests in Hashimoto's disease has varied considerably in all series reported in the literature. Although Morgans and Trotter (1957) and Murray and McGirr (1960) each found the test 100% positive in a small series of patients, Nilson and Berne (1964), Buchanan et al (1965) and Volpe et al (1965) who studied larger numbers of patients found only 48%, 30% and 38% positive tests respectively. The author's finding of a high incidence of positive IV perchlorate tests in patients with Hashimoto's disease would appear to confirm the uniform presence of defective utilisation of trapped iodide in this condition and indeed suggests that the IV test is more sensitive than the oral test. A further detailed comparison between techniques will be required to confirm this. It is likely however that the IV perchlorate test is the most discriminant of all radioactive iodine tests of thyroid function for diagnosis of Hashimoto's disease since other tests so often produce variable and conflicting results (Bastenie and Ermans, 1972).

It is of interest that the IV perchlorate test gave evidence of defective organification of thyroidal iodide in 19 out of 40 patients with simple goitre. Although this minor degree of dyshormonogenesis in simple goitre has been previously documented in vivo (Morgans and Trotter, 1957; Baschieri et al, 1963) and in vitro (Dimitriadou et

al, 1960), it remains problematical whether simple goitre develops because iodide organification is defective or whether the defect appears after goitre formation. It is unlikely that this defective iodide organification in patients with simple goitre could be explained by areas of focal thyroiditis (Buchanan et al, 1965) because serum antibody tests were negative. Indeed, it is debatable whether focal thyroiditis is causally related to defective iodide organification at all since none of the thyrotoxic patients previously studied (Chapter 11) showed a positive test despite the presence of high titres of serum anti-thyroid antibodies.

Considering the IV perchlorate test in isolation, it is clear that useful discrimination between Hashimoto's disease and simple goitre may be obtained by using 1.0% dose discharge as the 'significant' level rather than the experimentally derived normal discharge of 0.5% dose (Table 9). By this expedient, 80% of patients with Hashimoto's disease were positive but only 11.5% in the simple goitre group. Although this degree of separation between diseases is similar to that obtained using serum anti-thyroid antibodies in isolation (Doniach et al, 1960), it must be remembered that, whereas high titres of anti-thyroid antibodies in a euthyroid patient with a goitre are diagnostic of Hashimoto's disease, a discharge of $> 1.0\%$ dose can only imply the diagnosis of Hashimoto's disease since this functional abnormality could be the result of a number of separate congenital and acquired thyroid conditions.

Most clinicians will concede that diagnosis of Hashimoto's disease

can be made with confidence in euthyroid patients with non-toxic goitre when high titres of serum anti-thyroid antibodies are detected or when hypothyroidism is associated with the presence of medium or low titres. The problem in clinical practice arises when euthyroid patients with underlying thyroiditis have low titres of antibodies since differentiation from simple goitre may be difficult unless a thyroid biopsy is performed. It is likely that approximately 30% of patients with simple goitre and 10 - 20% patients with Hashimoto's disease fall into this category (Hall, 1962). In this study, a histological diagnosis was obtained in 5 such cases and in each subject, the IV perchlorate test predicted the correct diagnosis prior to thyroidectomy (Table 10). Accordingly, it is anticipated that the role of the IV perchlorate test in investigation of non-toxic goitre will be as an adjunct to diagnosis in euthyroid patients with low titres of anti-thyroid antibodies. Since the IV test incorrectly predicted the diagnosis in one of the two euthyroid patients with negative antibodies who discharged $>$ 1.0% dose, its precise role among these patients requires further clarification.

It would appear that by combining thyroid status, serum anti-thyroid antibody titres and the IV perchlorate test, a high degree of precision in diagnosis of Hashimoto's disease from simple goitre will be obtained which should obviate the need for thyroid biopsy in most cases. Biopsy remains mandatory if carcinoma of the thyroid is suspected in addition to Hashimoto's disease (Shands, 1960; Chesky et al, 1962). One patient with Hashimoto's disease who discharged

3.0% dose, had a co-existent carcinoma. Clearly a high index of suspicion is of paramount importance for this diagnosis.

In conclusion, the intravenous perchlorate test has a role in the definitive diagnosis of Hashimoto's disease and will be a useful adjunct in the differential diagnosis of non-toxic goitre.

SUMMARY - CHAPTER 13

In a study of patients with non-toxic goitre, the intravenous perchlorate discharge test indicated the presence of defective organification of thyroidal iodide in nearly all patients with Hashimoto's disease but in less than half those with simple goitre. Improved diagnostic discrimination was obtained by setting limits of significant discharge to at least 1% of administered ^{131}I dose. Using this criterion, 80% of patients with Hashimoto's disease but only 12% of patients with simple goitre showed a positive test. The intravenous perchlorate discharge test would appear of most value in clinical practice for the differential diagnosis of non-toxic goitre in those euthyroid patients who have low titres of serum anti-thyroid antibodies.

CHAPTER 14

DEFECTIVE IODIDE ORGANIFICATION IN THYROID FOLLOWING THERAPEUTIC IRRADIATION WITH RADIOACTIVE IODINE-131 OR 125

This chapter describes an in vivo study of iodide organification in thyroid following radioactive iodine therapy for thyrotoxicosis. The significance of the findings and their relevance to concepts of ^{125}I microdosimetry are discussed.

INTRODUCTION

Although many workers have diligently studied the therapeutic uses of radioactive iodine-131 (^{131}I), little attention has been devoted to disorders of thyroid physiology following such treatment. As described in Chapter 10, most in vivo studies of thyroid function after therapy have involved quantitation of thyroidal iodide transport by the technically simple measurement of early thyroidal ^{131}I uptake. A few researchers have studied thyroidal iodide organification in vivo after ^{131}I therapy (Kirkland, 1954; Larson, 1955; Keiffer et al, 1965) and have confirmed that defects of this mechanism can be radiation induced, but they provide conflicting evidence on both incidence and likely effect of the defect on the outcome of treatment.

In Chapter 10, it was proposed that a thyroid uptake of $^{99\text{m}}\text{Tc}$ could be used after therapy for sequential assessment of thyroid function. Since defects of iodide handling reduce the reliability of any measurement of iodide transport (i.e. $^{99\text{m}}\text{Tc}$ uptake) as an

index of total thyroid function, knowledge of the efficiency of this second step in hormone synthesis is clearly essential when ^{99m}Tc is used in this way. Accordingly, the intravenous perchlorate discharge test has been used to study this particular aspect of post-irradiation hormone synthesis.

Aims of the pilot study were twofold. Firstly, to assess the probable incidence of iodide organification defects following ^{131}I and ^{125}I therapy. Secondly, to determine whether the radiobiological effect on thyroidal iodide organification was dependent on the microdosimetry of the particular radionuclide used for treatment.

MATERIALS AND METHOD

Twenty seven patients were studied and in all, the clinical diagnosis of thyrotoxicosis was confirmed by routine radioactive iodine thyroid function tests and a PB ^{127}I . All patients were treated as out-patients and each had diffuse thyroid enlargement with an approximate thyroid weight of between 25 and 75 g. Twelve patients, all female, whose ages ranged from 36 to 55 years (45 ± 7 years = mean \pm LSD) were given thirteen therapy doses of ^{131}I (6.4 ± 2.8 mCi = mean \pm LSD). Fifteen patients; 2 male and 13 female, whose ages ranged from 39 to 62 years (46 ± 6 years = mean \pm LSD) were given fifteen therapy doses of ^{125}I (12.3 ± 4.4 mCi = mean \pm LSD). Thyroid function was measured with the intravenous perchlorate discharge test (Chapter 11) and a PB ^{127}I before and regularly after treatment.

Data from ^{131}I treated patients was obtained retrospectively; the ^{125}I study was prospective. In 6 patients treated with ^{125}I

TABLE 11

RESULTS OF IV PERCHLORATE DISCHARGE TEST IN PATIENTS

FOLLOWING RADIOACTIVE IODINE-131 THERAPY

PATIENT	WEEKS AFTER ¹³¹ I THERAPY				THYROID STATUS AT 48 WEEKS
	6 - 12	13 - 24	25 - 36	37 - 48	
1	++	neg	R	-	Euthyroid
2	-	neg	-	-	Euthyroid
3	++	-	R	-	Euthyroid
4	-	++	++	++	Euthyroid
5	neg	neg	H	neg	Euthyroid
6	-	neg	neg	neg	Thyrototoxic
7	neg	-	-	neg	Euthyroid
8	neg	-	-	neg	Thyrototoxic
9	neg	-	-	neg	Euthyroid
10	-	neg	neg	+	Euthyroid
11	-	neg	-	neg	Thyrototoxic
12	neg	neg	-	-	Thyrototoxic
13	neg	H	-	-	Euthyroid
14	++	neg	-	-	Euthyroid

R = Retreatment

H = Hypothyroid

Positive discharge (+) = 1.5 > Discharge > 0.5% dose

Strongly positive discharge (++) = Discharge > 1.5% dose

Negative discharge = neg

TABLE 12

RESULTS OF IV PERCHLORATE DISCHARGE TEST IN PATIENTS

FOLLOWING RADIOACTIVE IODINE-125 THERAPY

PATIENT	WEEKS AFTER ¹²⁵ I THERAPY					THYROID STATUS AT 48 WEEKS
	0 - 4	5 - 12	13 - 24	25 - 36	37 - 48	
1	-	++	++	neg	+	Thyrototoxic
2	+	+	+	R		
3	neg	neg	++	++	R	
4	-	neg	neg			
5	-	-	++	H		
6	-	neg	neg	+	neg	Thyrototoxic
7	neg	+	++	+	neg	Euthyroid
8	neg	++	+	neg	neg	Thyrototoxic
9	-	-	neg	-	neg	Thyrototoxic
10	neg	-	++	++	++	Euthyroid
11	neg	neg	neg			
12	neg	+	neg	neg	-	Thyrototoxic
13	neg	++	neg	++	-	Thyrototoxic
14	-	++	+	-	-	Euthyroid
15	-	-	++	neg	-	Thyrototoxic

R = Retreatment

H = Hypothyroid

Positive discharge (+) = 1.5 > Discharge > 0.5% dose

Strongly positive discharge (++) = Discharge > 1.5% dose

Negative discharge = neg

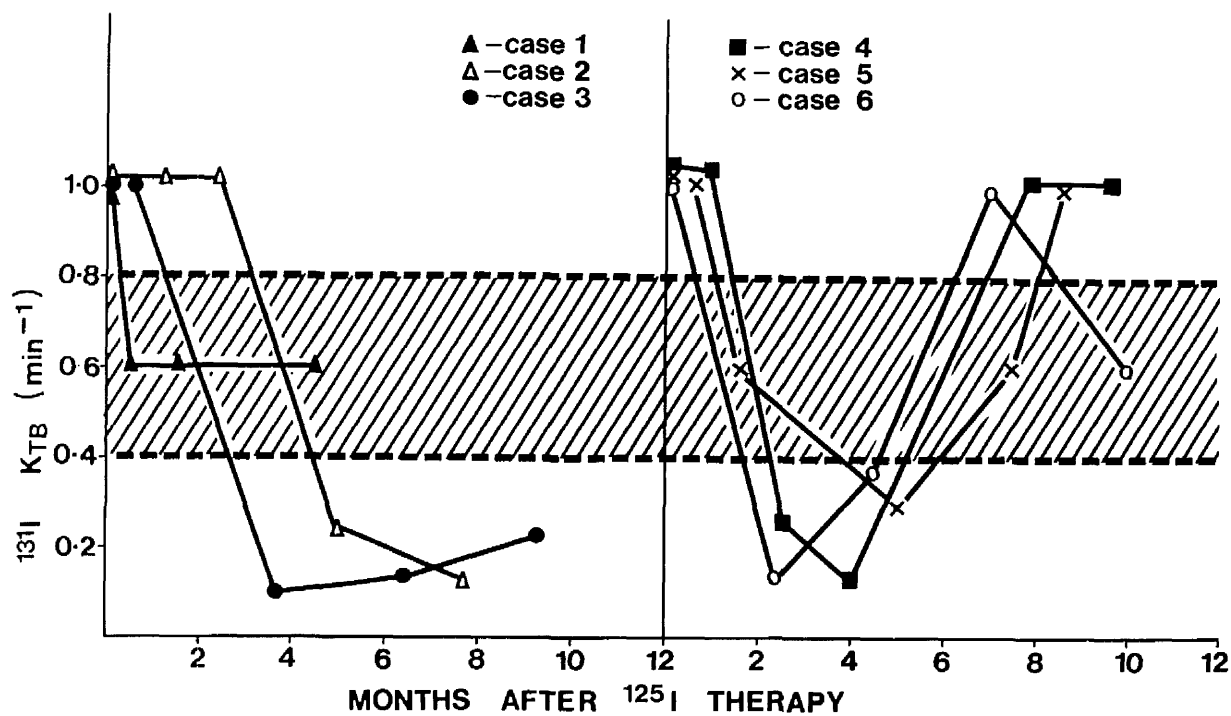


Figure 46: A serial calculation of the thyroidal ^{131}I organification-rate constant (K_{TB}) in 6 patients who demonstrated a defect of iodide organification in thyroid after radioactive iodine-125 therapy for thyrotoxicosis. When $0.40 < K_{\text{TB}} < 1.0$, it was arbitrarily placed on the graph at $K_{\text{TB}} = 0.60$.

where a defect of iodide organification was detected on more than two occasions, serial calculations of K_{TB} were made (Chapter 12).

It was assumed that secretion of organic ^{131}I from thyroid during the perchlorate test was negligible.

RESULTS

^{131}I Therapy

Data is presented in Table 11. There was a positive discharge test in 3 of 9 patients studied (33%) between 6 and 12 weeks after therapy but only 1 of nine studied (12%) between 13 and 24 weeks after therapy. Between 25 - 36 and 37 - 48 weeks after therapy, positive tests were seen in 1 of 3 (33%) and 2 out of 6 (33%) patients studied respectively.

In 1 patient (number 4), the defect of iodide organification has persisted for 18 months post therapy and she has remained euthyroid with a $FB^{127}I$ of approximately 8.6 ug/100 ml for over a year.

^{125}I Therapy

Data is presented in Table 12. In the first 4 weeks after therapy a weakly positive discharge was seen in 1 of 8 patients studied (12%); between 4 and 12 weeks, 7 out of 11 were positive (64%); between 13 and 24 weeks, 9 out of 15 were positive (60%); between 25 and 36 weeks, 5 out of 9 were positive (55.5%); and between 37 and 48 weeks, 2 out of 6 were positive (33%).

A serial calculation of K_{TB} (Chapter 12) in 6 patients with persistently positive tests is shown in Figure 46. Only 1 case (number 3) was undoubtedly euthyroid when the study ended. Since

K_{TB} calculation is accurate only up to a value of 0.40 min^{-1} , values of $1.0 > K_{TB} > 0.4$ were placed between the interrupted lines on the figure at $K_{TB} = 0.6 \text{ min}^{-1}$. Two main features were firstly, the general delay of some weeks after ^{125}I therapy before K_{TB} fell and secondly, a tendency for iodide organification to recover with time.

Statistical Analysis

The difference in incidence of defects of iodide organification between the ^{131}I and ^{125}I treatment groups was statistically significant only in the groups studied 13 - 24 weeks after therapy. At this time, $11.2 \pm 10.5\%$ (proportion \pm SE of proportion) of patients treated with ^{131}I showed a defect and $60.0 \pm 13.2\%$ (proportion \pm SE of proportion) of patients treated with ^{125}I showed a defect. The SE of difference between the groups was 16.8%. This observed difference was approximately equal to 3 x its SE and $P = .01$.

DISCUSSION

Data collected retrospectively from patients treated with ^{131}I indicates that defects of thyroidal iodide organification do not commonly occur following therapeutic use of this radionuclide. This is in agreement with the findings of Larson (1955) but conflicts with the report of Keiffer et al (1965).

The more comprehensive data from the prospective study of patients treated with radioactive iodine-125 (^{125}I), allow some generalisations to be made.

1. The post-irradiation defect of iodide organification is uncommon in the first month after therapy.

Comparison of ^{131}I and ^{125}I

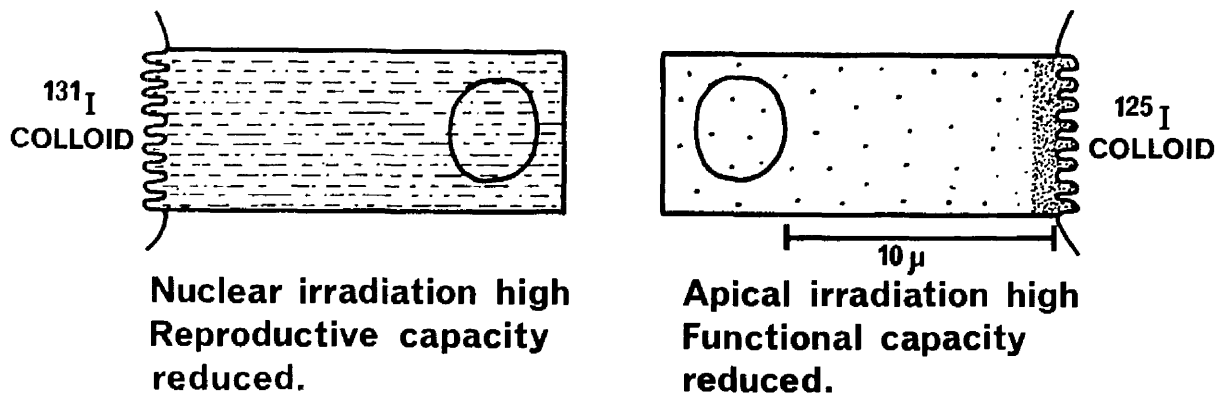


Figure 47: Diagrammatic representation of the hypothetical microdosimetry of ^{131}I and ^{125}I across thyrotoxic follicular cells. Whereas the penetrating electrons from ^{131}I irradiate both follicular cell cytoplasm and nucleus, ^{125}I tends to selectively irradiate the colloid-cell interface where iodide organification occurs with a relative sparing of the cell nucleus.

2. Thereafter, the defect is common and most often appears between 1 and 4 months after therapy.
3. There appears no consistent relationship between the presence of a defect and any particular thyroid status. Surprisingly some patients remained clearly thyrotoxic despite a major defect of iodide organification.

Notwithstanding the small number of patients studied, it is clear that ^{125}I therapy is more likely to reduce the efficiency of thyroidal iodide organification than treatment with an equivalent dose of ^{131}I . This important observation will be seen to have theoretical and practical relevance to concepts of and rationale for, ^{125}I therapy.

Nuclei of thyroid follicular cells have been shown in vitro (Hall and Grand, 1962) and ⁱⁿvivo (Greig, 1965) to be more radiosensitive than those areas of cell where hormone synthesis occurs. The radiation dose required to impair hormone synthesis will therefore tend to create severe nuclear damage with resulting cell sterility or death. This sequence of events is the likely prelude to post ^{131}I therapy hypothyroidism (Greig, 1965), and is thought to result from uniform irradiation of thyroid cells and stroma from energetic β emissions of ^{131}I (Figure 47). In contrast, theoretical calculations (Gillespie et al, 1970) have indicated that emitted electron radiations from ^{125}I have a low penetrating potential. The therapy dose will therefore exert a greater radiobiological effect on the apical margin of cell (Figure 47) and hormone synthesis than on the nucleus and basal surface (iodide transport). Such theoretical considerations imply that the special microdosimetry of ^{125}I will allow both

satisfactory control of thyrotoxicosis and relative freedom from subsequent thyroid failure because of a proportionate decrease in nuclear damage. Confirmatory data on ^{125}I microdosimetry has been obtained from elegant animal studies (Gross et al, 1968; Greig et al, 1970). This observation that ^{125}I therapy reduces efficiency of iodide organification more commonly than ^{131}I therapy extends such supportive data at a clinical level.

A further interesting feature in the ^{125}I treated patients is a delay of several weeks before this particular dysmorphonogenesis is detected. This suggests that the phenomenon is related to total gland radiation rather than therapy dose-rate.

These patients have been studied for up to 1 year after ^{125}I therapy. Although the long term effect of this particular defect is conjectural, it would seem likely that those patients who develop major defects of iodide organification, will be more sensitive than normal to further therapeutic irradiation.

When more data from treated patients is available, firmer conclusions on the relative incidence and therapeutic significance of defective iodide organification will be possible.

SUMMARY ↔ CHAPTER 14

The intravenous perchlorate discharge test was performed in a small series of thyrotoxic patients at intervals up to 1 year after radioactive iodine-131 (^{131}I) or -125 (^{125}I) therapy, to define the radiobiological effect of each radionuclide on thyroidal iodide organification. Organification dyshormonogenesis was more commonly detected following ^{125}I therapy and this difference between radionuclides was highly significant between 3 - 6 months after therapy when 60% of patients given ^{125}I but only 11% of patients given ^{131}I demonstrated the abnormality. These results are totally consistent with a current hypothesis on ^{125}I microdosimetry in which it is believed that the apical membrane of thyroid follicular cell, site of iodide organification, receives more deposited radiation than the nucleus and basal cell membrane.

CHAPTER 15

FAMILIAL GOITROUS HYPOTHYROIDISM:

INVESTIGATION OF THREE SIBLINGS

This chapter presents an unexplained remission of hypothyroidism in one of three brothers who each had a defect of thyroidal iodide organification.

INTRODUCTION

A well documented clinical and biochemical diagnosis of hypothyroidism is usually correctly accepted as indicating the need for life-long thyroxine therapy unless the patient has received a drug which is known to interfere with thyroxine synthesis. Such drugs include carbimazole, iodide (Begg and Hall, 1963), para-aminosalicylate (McGregor and Sommer, 1954) and resorcinol (Bull and Fraser, 1950). In these circumstances, withdrawal of the offending drug results in a remission of hypothyroidism.

The author has had an opportunity to study and here reports a remission of clinical and biochemical hypothyroidism which occurred in one of three brothers each of whom presented with goitrous hypothyroidism.

CASE MATERIAL

Methods

Case 1 (G.B.) This patient presented to Dr. J.A. Thomson, Royal Infirmary, aged 19 years in 1966. He was clinically hypothyroid with an enlarged thyroid gland x 2. Hypothyroidism was confirmed

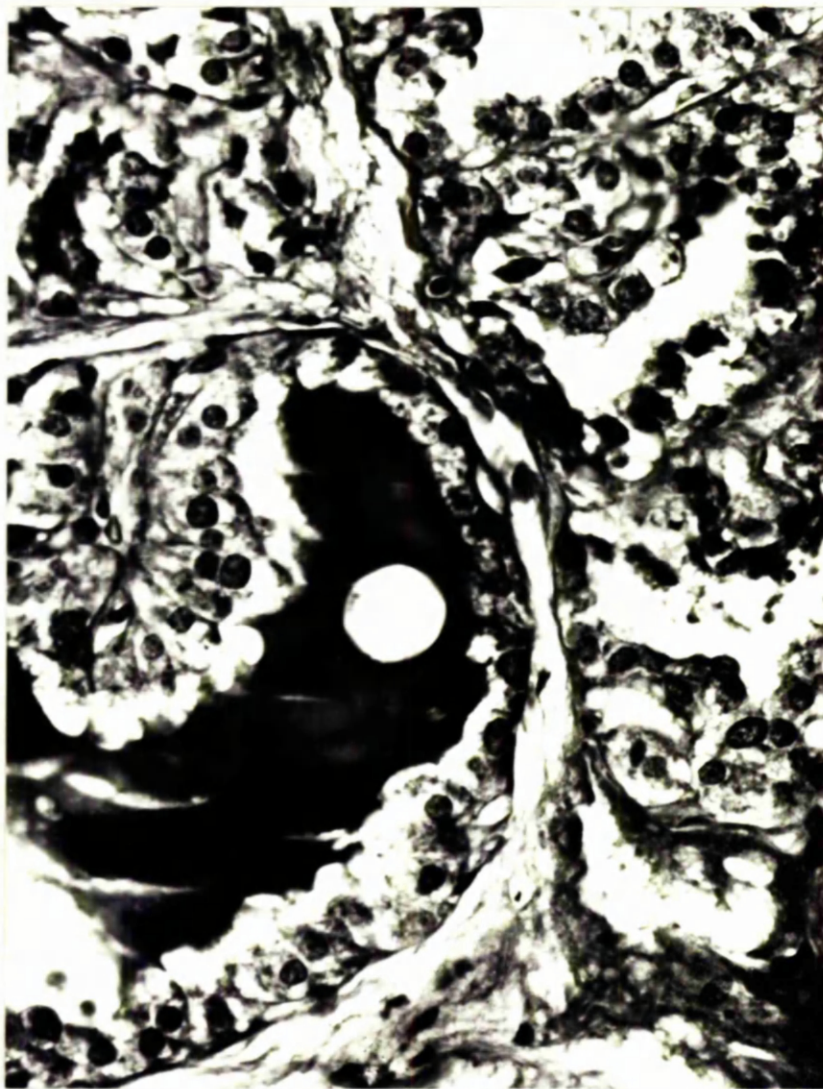


Figure 48: Histology of thyroid in case 1. G.B. (x 560).
The follicular cells show evidence of TSH stimulation
but thyroiditis is not present.

TABLE 13

Data on Case 1. G.B.

Time after stopping thyroxine	¹²⁷ I PB ug/100 ml	Net clearance of ¹³¹ I by the thyroid (ml/min)				I.V. Perchlorate Discharge Test
		0 - 5	5 - 10	10 - 15	15 - 20	
4 weeks	-	-	150	103	97	-
20 weeks	5.8	-	64	25	29	-
46 weeks	6.1	-	48	39	38	1.6% dose 21% gland uptake
16 months	10.1	-	-	-	-	1.5% dose 19% gland uptake
28 months	8.2	-	-	-	-	1.8% dose 15% gland uptake
40 months	1.5	-	-	-	-	4.9% dose 50% gland uptake

with a PB ^{127}I of 0.3 ug% and a BMR 47% below standard. The thyroid uptake at 4, 24 and 48 hours after oral ^{131}I was 17, 15 and 12% dose respectively and a 48 hour PB ^{131}I was negligible. The salivary : plasma ratio of ^{131}I was 16 : 1 and a conventional oral perchlorate discharge test was negative. A thyroid biopsy showed a hyperplastic gland without evidence of thyroiditis (Figure 48) and a precipitin test was negative. An oral MIT test revealed normal deiododination activity in body tissues thus excluding a dehalogenase defect in thyroid.

A diagnosis of probable dyshormonogenetic goitre was made, although he did not fit into any of the typical defect patterns, and thyroxine therapy was instituted. On thyroxine, signs of hypothyroidism disappeared and the thyroid gland became impalpable.

In May, 1970, at the author's invitation, the patient agreed to discontinue thyroxine to permit further study. Subsequent investigations are shown in Table 13. One month after stopping thyroxine, his early thyroid net clearance of ^{131}I (Chapter 8) was elevated and showed a 30% reduction in the 15 - 20 minute period as compared to the 5 - 10 minute period; this was consistent with the presence of a defect of iodide organification (Owen et al, 1960). Reassessment at 20 and 46 weeks after stopping thyroxine revealed firstly, a reduction in absolute net clearance of ^{131}I with continued evidence for defective iodide organification and, secondly, unequivocal euthyroidism. At 46 weeks, the IV perchlorate test (Chapter 11) confirmed defective iodide organification with a discharge of 1.6%

T A B L E 1 4

Data on Case 2. W.B.

Time after stopping thyroxine	¹²⁷ I PB ug/100 ml	Net clearance of ¹³¹ I by the thyroid (ml/min)				I.V. Perchlorate Discharge Test
		0 - 5	5 - 10	10 - 15 (min)	15 - 20	
4 weeks	-	-	88	79	80	-
20 weeks	3.0	-	59	28	9	1.6% dose 25% gland uptake
24 weeks	1.0	111	27	0	0	-

dose. Similar results were obtained at 16 and 28 months after stopping thyroxine, his $PB^{127}I$ remaining approximately 8.2 ug%. The serum TSH, estimated at 28 months by courtesy of Professor Hall, Newcastle, was 2.1 u units/ml (normal range = 0.5 - 3.5 u units/ml). He remained asymptomatic but when reviewed 40 months after stopping thyroxine, the intravenous perchlorate test revealed an increased discharge of 4.9% administered dose. A total serum thyroxine of 2.6 ug/100 ml, serum triiodothyronine of 1.9 ng/ml and serum TSH of 33 units/ml, all measured by Dr. Ratcliffe, Glasgow, confirmed that a relapse of hypothyroidism was imminent.

Case 2 (W.B.) This boy, brother of case number 1, presented to Dr. M.J. Riddell, Victoria Infirmary, Glasgow in 1959, aged 11. He was clinically hypothyroid with an enlarged thyroid gland x 2. BMR was 34% below standard and serum anti-thyroid antibodies (precipitin test and CF tests) were negative. Thyroxine was commenced but treatment lapsed the following year. In 1965, when again clinically hypothyroid, he presented to Dr. J.A. Thomson, Royal Infirmary, Glasgow for investigation, $PB^{127}I$ at this time was 0.1 ug% and a conventional oral perchlorate discharge test was negative. He was recommenced on thyroxine and soon became euthyroid.

In May, 1970, like his brother, he agreed to discontinue thyroxine to allow further study. Subsequent investigations are shown in Table 14. Four weeks after stopping thyroxine, his thyroid net clearance of ^{131}I was in the high normal range and showed little reduction in the 5 - 20 minute period. Five months after stopping thyroxine,

TABLE 15

Data on Case 3, R.B.

Time	¹²⁷ I PB ug/100 ml	Net clearance of ¹³¹ I by the thyroid (ml/min)				I.V. Perchlorate Discharge Test
		0 - 5	5 - 10	10 - 15	15 - 20	
			(min)			
Before thyroxine therapy 1970	5.0	380	100	42	0	-
Thyroxine stopped for 6 weeks 17 months after starting therapy	6.5	180	170	112	105	5.2% dose 43% thyroidal uptake

however, he was clinically mildly hypothyroid with a PB^{127}I of 3.0 ug% and a marked reduction in net clearance of ^{131}I was detected in the 5 - 20 minute period indicating defective iodide organification. Confirmation of this defect was obtained with the IV perchlorate test which demonstrated a discharge of 1.6% dose. Treatment was withheld for a further 4 weeks but since hypothyroidism became more obvious clinically, thyroxine therapy was restarted.

Case 3 (R.B.) The third brother, aged 17 years, was first seen in April, 1970 by Dr. M.J. Riddell, Victoria Infirmary, Glasgow when he presented with goitrous hypothyroidism. On subsequent referral to the Thyroid Clinic, Royal Infirmary, Glasgow for further investigation, he was found clinically to be grossly hypothyroid with a thyroid gland enlarged x 3. Although a PB^{127}I at this time was 5.0 ug% the 24 hour uptake of ^{131}I was 18% dose and PB^{131}I was negligible. Thyroid precipitin test was negative. Detailed investigations are shown in Table 15. The ^{131}I clearance studies showed the classical pattern of defective iodide organification; high initial value and subsequent reduction. Thyroxine therapy was commenced with dramatic clinical improvement and disappearance of his goitre.

Seventeen months after commencing thyroxine, treatment was withheld for 6 weeks to allow further assessment. At the time of this study, PB^{127}I was 6.5 ug% and the ^{131}I net clearance revealed a 30% reduction over the period 5 - 20 minutes. An IV perchlorate discharge test confirmed a defect of iodide organification with a

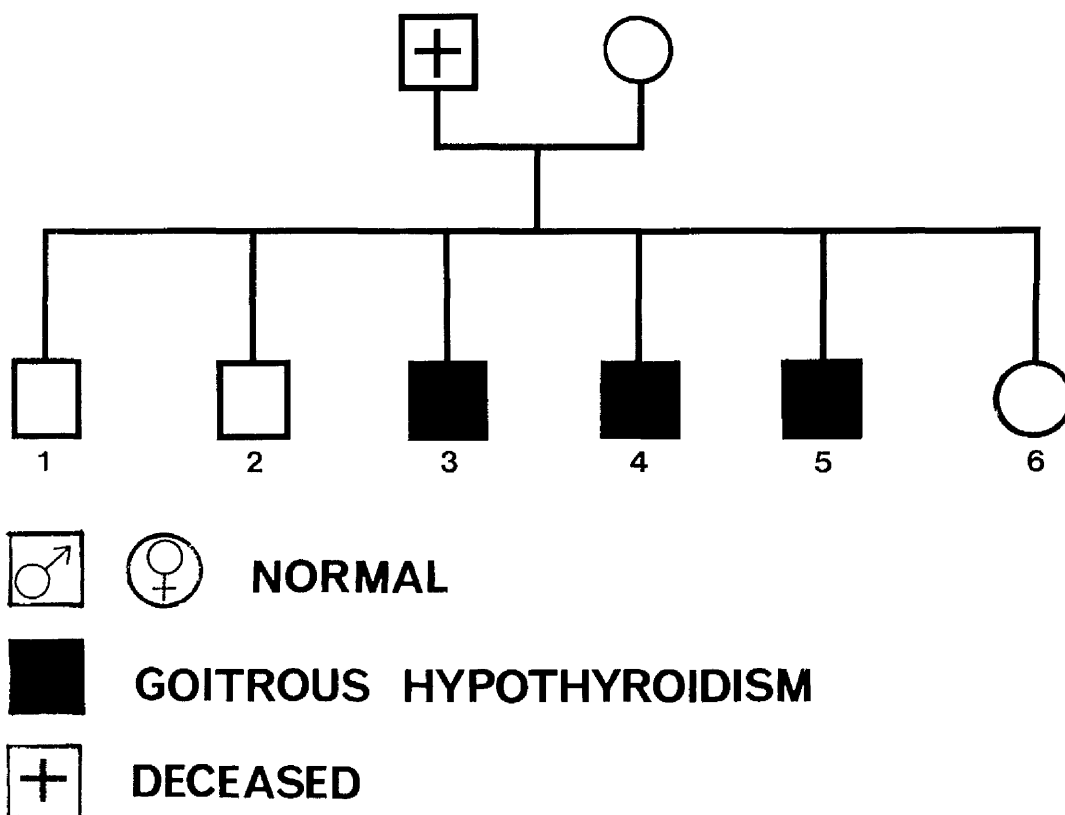


Figure 49: Family history: case 1 (G.B.) is sibling 3,
 case 2 (W.B.) is sibling 4 and case 3 (R.B.) is
 sibling 5.

discharge of 5.2% dose from the gland. Although the patient was euthyroid 6 weeks off therapy, he informed us that he was contemplating emigration. Because of uncertainty about his remaining in the Glasgow area, thyroxine therapy was recommenced.

OTHER MEMBERS OF THE FAMILY

The family tree is shown in Figure 49. There is no evidence of consanguinity. The parents had no clinical evidence of thyroid disease nor do other sibs. There is no history of deafness in any members of the family and there is no previous history of thyroid disease in other relatives.

DISCUSSION

These three brothers appear to have a dyshormonogenetic goitre due entirely or in part to defective organification of iodide within the thyroid. It is of particular interest that conventional perchlorate tests failed to reveal the defect while the intravenous perchlorate test clearly demonstrated the abnormality.

Since the brothers had a similar biochemical defect, it is difficult to understand why they showed such variation in clinical response to cessation of thyroxine therapy. While it is easy to understand in case 2 why discontinuing thyroxine resulted in the rapid restoration of hypothyroidism, the outcome in case 1 could not have been predicted. He had been clinically and biochemically hypothyroid when first seen and had shown an excellent clinical response to thyroxine treatment. Stopping this treatment did not cause a relapse of hypothyroidism over a 3 year period despite continuance of the biochemical defect. Indeed his plasma TSH level at 28 months off

thyroxine was in the normal range suggesting strongly that his thyroid was not under TSH stimulation which would have been expected if even mild hypothyroidism had been present (Evered et al, 1973). When hypothyroidism eventually re-appeared after 3 years the biochemical defect became more obvious on testing and his plasma TSH rose.

Although no similar reports have appeared in the literature, Zondek et al (1960) have documented a comparable phenomenon. These workers administered large doses of thyroid hormones in the short term (a form of treatment referred to as Stoss therapy) to three hypothyroid siblings, each with a defect of thyroidal iodide organification and produced a protracted clinical remission of hypothyroidism for 6 months in 2 and $3\frac{1}{2}$ years in the third. In contrast to our own findings, however, they were unable to produce the remission with standard thyroxine replacement therapy. Zondek's postulate that Stoss therapy possibly activated dormant enzymatic pathways in the thyroid was criticised by Stanbury (1961) on the basis firstly that it was impossible to exclude such factors as the surreptitious administration of thyroxine and secondly, that Zondek had failed to exclude a dehalogenase defect which would have responded to the iodide given in 'Stoss therapy'. We are satisfied that in our patients, there was no surreptitious intake of thyroxine and in case 1, the persistent moderately elevated radioactive iodine clearance of the thyroid would be against any self-medication. In addition we have shown that the MIT test was normal in case 1. It could also be argued that in 1965, the cause of hypothyroidism was the presence of severe iodine deficiency

in conjunction with a sub-clinical defect of thyroidal iodide organification. This appears unlikely since Glasgow is not an iodine deficient area and our patients' diets have not apparently varied over the last 7 years.

Although it can be shown that the defect of iodide organification in thyroid can vary in severity among affected patients with the disorder (Dax and Weiner, 1967), it is difficult to understand why, in a single patient (case 1), the expression of the defect should vary from time to time. One must postulate that something occurred during thyroxine treatment to increase the efficiency of endogenous thyroxine formation and secretion but whether this was in response to the physiological replacement of thyroxine or whether it indicates a real spontaneous variation in the disease is a matter for conjecture.

SUMMARY - CHAPTER 15

Three male siblings with familial goitrous hypothyroidism due to a defect of thyroidal iodide organification have been studied. Withdrawal of thyroxine therapy from two of the brothers led to a rapid return of clinical hypothyroidism in one but the other brother remained euthyroid for approximately 3 years off his thyroxine therapy despite continuing evidence of defective iodide organification in his thyroid. The phenomenon is contrasted with that produced by massive administration of thyroid hormones (Stoss therapy), but no satisfactory explanation for its occurrence can be given.

SECTION 5

SUMMARY AND CONCLUSIONS

Chapter 16

Summary and conclusions

CHAPTER 16

SUMMARY AND CONCLUSIONS

In recent years, it has become increasingly apparent that the in vitro measurement of plasma hormones concerned in thyroid homeostasis provides an ideal basis for the clinical determination of thyroid status. Although the well known in vivo tests of thyroid function using radioactive iodine have thereby been relegated to a role of secondary importance, the widespread introduction of sophisticated imaging devices has generated renewed interest in the in vivo approach to thyroid investigation. Rapid changes in thyroidal radioactivity may be accurately analysed with ease using this equipment and the resulting information on the kinetic behaviour of thyroid radionuclides can provide unique information on different aspects of thyroid function.

In view of the potential importance of thyroid kinetic studies in the understanding, investigation and treatment of human disease, the author's objectives in this thesis were threefold. Firstly, since a scintillation camera was not available, to develop a directional counting system which could provide comparable quantitative data on rapidly changing thyroid radioactivity. With this system, and accepting contemporary precepts on thyroidal iodide kinetics, the second aim was to study the thyroid physiology of pertechnetate- ^{99m}Tc (^{99m}Tc) in health and disease ostensibly to define its precise role in thyroid investigation but also to explore areas

of therapeutic potential. Thirdly, to develop a sensitive index of iodide organification in thyroid which could be applied to investigate thyroid utilisation of iodide in normal subjects and in various congenital and acquired human thyroid diseases.

The directional counting system for continuous recording of thyroid radioactivity consisted of a closely collimated scintillation detector connected by scaler and ratemeter to an automatic recorder. The collimator, by design, considerably reduced the contribution of extrathyroidal radioactivity to total neck radioactivity thereby allowing more precise measurement of thyroid radioactivity.

A significant arterio-venous difference in plasma ^{99m}Tc and iodide- ^{131}I concentration, noted at early intervals following their intravenous injection, was recognised to be a major systematic error of early phase kinetic studies of thyroid and an approximation to minimise this was adopted.

Following an account of autoradiographic studies with ^{99}Tc in rat thyroid which confirmed the relevance of contemporary model theory to kinetics of pertechnetate in the thyroid, the thesis continued with studies to define the role of ^{99m}Tc in clinical investigation and treatment of human thyroid disease. Using the adapted directional counting system, quantitation of thyroid transport of ^{99m}Tc and ^{131}I in euthyroid and thyrotoxic subjects revealed direct and equal proportionality between thyroid unidirectional clearance (U.D.C.) of ^{99m}Tc and both unidirectional and net clearance of ^{131}I . This result indicated that, although time consuming, measurement of

the thyroid U.D.C. of ^{99m}Tc gave a precise index of iodide transport in thyroid unaffected by disorders of iodide organification. Thyroid uptake of ^{99m}Tc , a simple alternative index of iodide transport, was found ideal for routine clinical studies and valuable for diagnosis of thyrotoxicosis. Since variation in the thyroid exit-rate constant (K_{TP}) between patients could occasionally produce inaccurate values, it was concluded that this measurement was most suitable for sequential assessment of iodide transport in the same patient during some therapeutic manoeuvre.

Pursuing thyroid physiology, evidence was provided that the thyroid exit rate-constant (K_{TP}) was a result of simple ionic diffusion from functional follicles. The close correlation between exit rate-constants of ^{99m}Tc and ^{131}I , both before (K_{TP}), and after perchlorate (K'_{TP}), further indicated a similar if not identical diffusion pathway from thyroid for each radionuclide. In addition to a blocking action on thyroid transport of ^{99m}Tc and ^{131}I , perchlorate magnified this diffusion by a factor of 2 to 3.

The thesis continued with an account of one possible application for ^{99m}Tc uptake measurements in human therapeutics, namely sequential assessment of thyroid function after radioactive iodine therapy to predict the adequacy of such treatment. Therapy with ^{125}I was clearly shown to affect thyroid iodide transport in a biphasic manner with an immediate reversible effect, possibly dose-rate related, and a delayed irreversible effect, possibly proportional to the total deposited radioactivity. As a result, the thyroid uptake of ^{99m}Tc

had most prognostic value 4 - 6 months after ^{125}I treatment. Similar conclusions broadly applied to ^{131}I therapy although this was not studied in comparable detail.

Following a change of theme, the thesis expanded the concepts of iodide organification in thyroid in relation to radioactive iodide kinetics and continued by describing in detail a new technique for detecting unorganified (inorganic) thyroidal ^{131}I . This intravenous perchlorate discharge test, developed with particular reference to kinetic behaviour of inorganic iodide in thyroid, was shown to be simple and efficient. Inorganic iodide was not detected in euthyroid or thyrotoxic human subjects though moderate amounts were found in normal rat thyroid. These findings indicated a basic difference in thyroid iodide metabolism between the species and argued that whereas iodide transport was rate-limiting for thyroxine synthesis in humans, iodide organification was rate-limiting in normal rats.

To further define the utilisation of trapped iodide by human thyroid in vivo, the organification rate (K_{TB}) of ^{131}I was calculated in patients with organification disorders by means of a theoretical analysis developed with the aid of analogue computation. Although variation in inorganic iodide content of thyroid between patients rendered the index (K_{TB}) valueless for routine clinical use, its sequential measurement in the same patient following radioactive iodine therapy illustrated the faulty utilisation of trapped iodide which may be present for a variable time after treatment.

Application of the intravenous perchlorate test to the differential diagnosis of non-toxic goitre revealed inorganic ^{131}I in the thyroid of all patients with Hashimoto's disease but also in about half those with simple goitre. It was concluded that the test was a useful adjunct to diagnosis in those euthyroid patients with low titres of serum anti-thyroid antibodies. The author was unable to evaluate the relative importance and contribution of defective iodide organification to the pathogenesis of simple goitre.

The thesis continued with a study to define the radiobiological effect of treatment with radioactive iodine- ^{131}I and ^{125}I on iodide organification in thyroid. Faulty utilisation of trapped iodide followed ^{125}I more commonly than ^{131}I therapy and this difference between radionuclides was totally consistent with prevailing views on their microdosimetry in thyroid.

The section ends with the account of an unexplained remission of hypothyroidism in one of three brothers each of whom had a defect of iodide organification in thyroid.

This thesis has drawn heavily on the store of thyroid model theory developed over the last two decades from small animal studies. Although there are subtle differences in thyroid physiology between species, it is clear that these thyroid models are relevant to the human situation and assist our understanding of some complex biochemical events in thyroid hormone synthesis.

Kinetic studies of thyroidal iodide metabolism currently play a minor role in the routine investigation and treatment of thyroid

disorders but clearly, the information obtained from their use may be equally of value in a clinical or research context. By indicating the scope of such studies and establishing areas for future development, the author has underlined the clinical importance of these concepts of kinetic analysis which were only recently introduced into the study of human thyroid disease.

APPENDIX 1

SUPPORTING TABLES

TABLE A

RESULTS OF KINETIC ANALYSIS FOR ^{131}I AND $^{99\text{m}}\text{Tc}$

USING THE TWO-COMPARTMENT THYROID MODEL:

MEASUREMENT IN PATIENTS WITH A BLOCK TO

THYROIDAL IODIDE ORGANIFICATION

<u>PATIENT</u>	<u>GRAPHICAL/DIGITAL ANALYSIS</u>			
	<u>$^{99\text{m}}\text{Tc}$</u>		<u>^{131}I</u>	
	<u>C L/min</u>	<u>$K_{TP} \text{ min}^{-1}$</u>	<u>C L/min</u>	<u>$K_{TP} \text{ min}^{-1}$</u>
1	0.106	0.116	0.210	0.086
2	0.073	0.140	0.179	0.099
3	0.072	0.036	0.184	0.047
4	0.076	0.064	0.133	0.030
5	0.055	0.079	0.115	0.073
6	0.064	0.087	0.156	0.068
7	0.071	0.140	0.216	0.106
8	0.117	0.054	0.252	0.044
9	0.095	0.125	0.155	0.052
10	0.093	0.097	0.125	0.053
11	0.096	0.046	0.278	0.034
12	0.051	0.067	0.126	0.060
13	0.024	0.045	0.060	0.040
14	0.082	0.140	0.158	0.079

CORRELATION BETWEEN THYROIDAL EXIT-RATE CONSTANT
OF ^{99m}Tc BEFORE (K_{TP}) AND
CORRELATION BETWEEN THYROIDAL EXIT-RATE CONSTANT
OF ^{99m}Tc BEFORE (K_{TP}) AND
AFTER PERCHLORATE (K'_{TP})

<u>PATIENT</u>	<u>^{99m}Tc</u>	
	<u>K_{TP} (min^{-1}) = x</u>	<u>K'_{TP} (min^{-1}) = y</u>
1	0.260	0.67
2	0.105	0.24
3	0.094	0.38
4	0.080	0.28
5	0.053	0.25
6	0.140	0.44
7	0.150	0.39
8	0.100	0.55
9	0.140	0.38
10	0.290	0.55
11	0.060	0.24
12	0.125	0.53
13	0.093	0.40
14	0.088	0.42
15	0.125	0.60
16	0.090	0.50

$$r = 0.64$$

$$y = 1.32x + 0.26$$

70% Confidence Limits

Gradient. 1.77 and 0.87 ($\pm 34\%$).

Intercept. 0.32 and 0.20 ($\pm 24\%$).

TABLE C

CORRELATION BETWEEN THYROIDAL EXIT-RATE CONSTANT
OF ^{131}I BEFORE (K_{TP}) AND
AFTER PERCHLORATE (K'_{TP})

<u>PATIENT</u>	^{131}I	
	<u>K_{TP} (min^{-1}) = x</u>	<u>K'_{TP} (min^{-1}) = y</u>
1	0.088	0.20
2	0.110	0.20
3	0.045	0.15
4	0.039	0.12
5	0.075	0.14
6	0.070	0.16
7	0.050	0.20
8	0.050	0.18

$$r = 0.52$$

$$y = 0.67x + 0.12$$

70% Confidence Limits

Gradient.1.16 and 0.172 ($\pm 74\%$).

Intercept.0.16 and 0.09 ($\pm 27\%$).

TABLE D

MEASUREMENT OF THYROID UNIDIRECTIONAL CLEARANCE
OF ^{99m}Tc : CORRELATION BETWEEN GRAPHICAL/
DIGITAL AND ANALOGUE METHODS OF ANALYSIS

<u>PATIENT</u>	<u>^{99m}Tc UNIDIRECTIONAL CLEARANCE</u>	
	<u>ANALOGUE (x)</u>	<u>GRAPHICAL/DIGITAL (y)</u>
1	0.240	0.265
2	0.347	0.188
3	0.114	0.073
4	0.071	0.066
5	0.022	0.017
6	0.076	0.063
7	0.147	0.097
8	0.104	0.065
9	0.072	0.060
10	0.220	0.220

$$r = 0.86$$

$$y = 0.71x + 0.01$$

70% Confidence Limits

Gradient.0.88 and 0.55 ($\pm 23\%$).

Intercept.0.035 and - 0.017 ($\pm 270\%$).

TABLE E

MEASUREMENT OF THYROID EXIT-RATE CONSTANT
OF ^{99m}Tc : CORRELATION BETWEEN GRAPHICAL/
DIGITAL AND ANALOGUE METHODS OF ANALYSIS

<u>PATIENT</u>	<u>^{99m}Tc EXIT-RATE CONSTANT (K_{TP})</u>	
	<u>ANALOGUE (x)</u>	<u>GRAPHICAL/DIGITAL (y)</u>
1	0.150	0.170
2	0.123	0.072
3	0.210	0.140
4	0.071	0.068
5	0.098	0.069
6	0.067	0.067
7	0.122	0.085
8	0.180	0.110
9	0.124	0.097
10	0.200	0.180

$$r = 0.80$$

$$y = 0.69x + 0.12$$

70% Confidence Limits

Gradient 0.89 and 0.496 ($\pm 28\%$).

Intercept 0.041 and - 0.016 ($\pm 231\%$).

APPENDIX 2

STANDARD METHODS

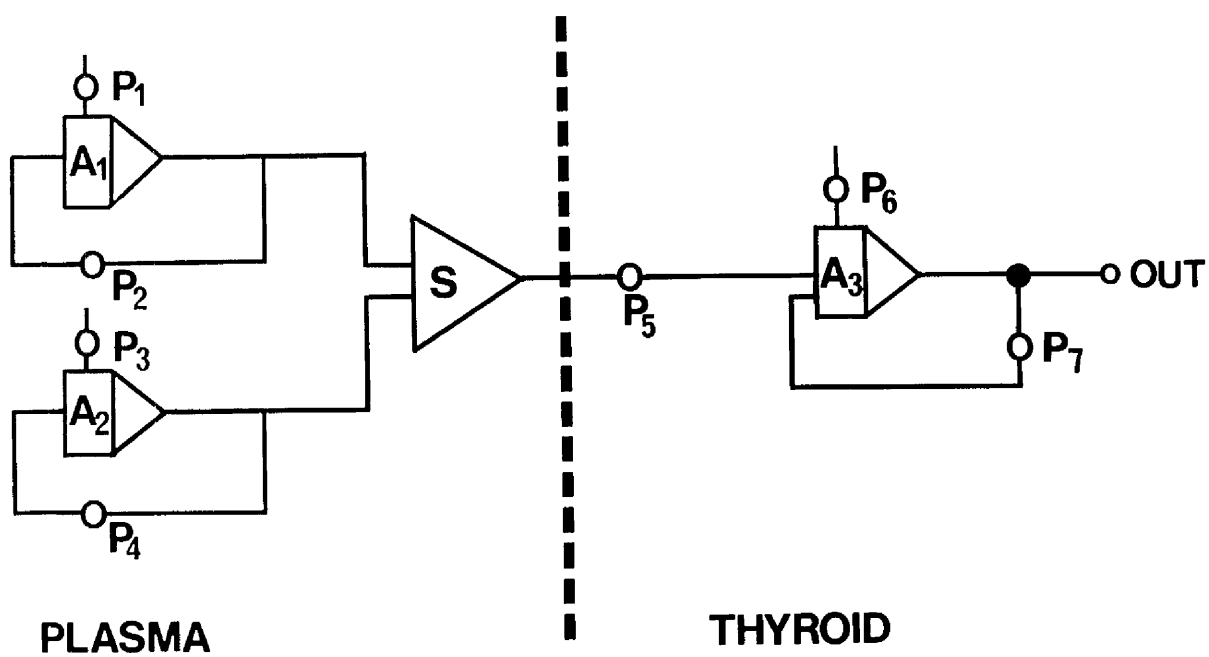


Figure 50: Block diagram of analogue computer circuit for a two-compartment thyroid model.

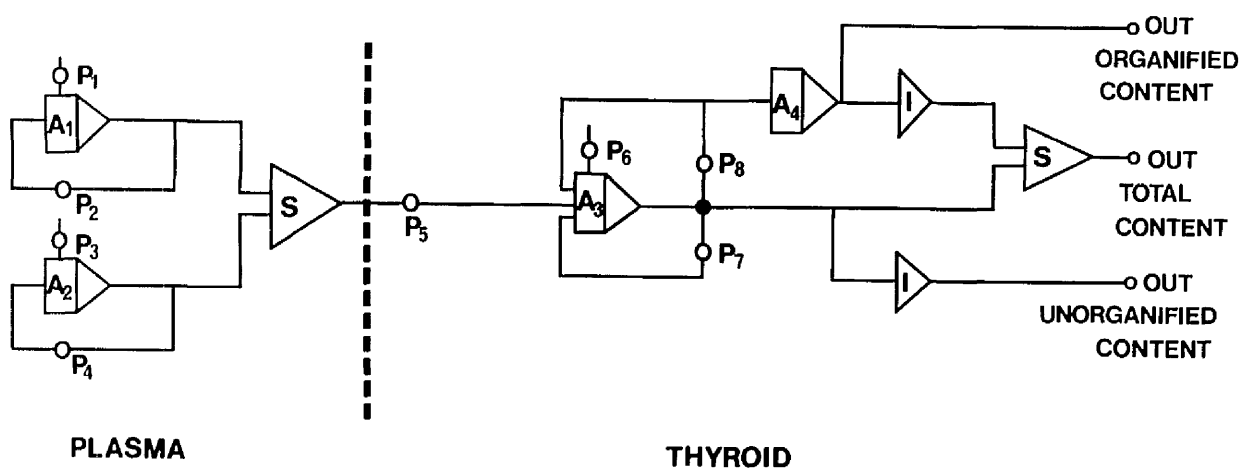


Figure 51: Block diagram of analogue computer circuit for a three-compartment thyroid model.

APPENDIX 2

STANDARD METHODS

ANALOGUE COMPUTATION USING TWO-COMPARTMENT MODEL

The block diagram of the circuit for a two-compartment thyroid model is shown in Figure 50.

Assuming bi-exponential decay for plasma radioactivity (^{99m}Tc or ^{131}I), P_1 and P_3 are adjusted to the predicted plasma concentration (fract. dose/l) at zero time. Suitable adjustment to the decay rate of the first and second exponential term are made using P_2 and P_4 until the output of the summing amplifier corresponds to the measured decay curve of plasma radionuclide over 20 minutes. Time scale was 1 min/second.

A fraction of output of S is selected by P_5 , representing thyroid unidirectional clearance, and transferred to the input of integrator A_3 with P_6 set at zero. A fraction of output of A_3 is subtracted by P_7 , representing K_{TP} , and the resulting total output is equivalent to thyroid content of radionuclide as a function of time. The values of P_5 and P_7 are adjusted until the best correspondence between model output and actual data on thyroid uptake is obtained. The values of P_5 (unidirectional clearance of thyroid) and P_7 (K_{TP} of thyroid) may then be read from the potentiometers.

ANALOGUE COMPUTATION USING THREE-COMPARTMENT MODEL

The block diagram of the circuit for a three-compartment model is shown in Figure 51. It comprises the basic two-compartments with

an additional integrating amplifier (A_4) as the third or organified ^{131}I compartment.

The plasma generator is adjusted to plasma curve of ^{131}I as before. A fraction of output of S_1 is selected by P_5 (unidirectional clearance) and transferred to the input of integrator A_3 with P_6 set at zero. Two fractions are subtracted from output of A_3 , which represents thyroid content of unorganified ^{131}I . Firstly, the fraction subtracted by P_7 representing diffusion via K_{TP} and secondly, subtraction by P_8 representing organification via K_{TB} . This fraction of unorganified ^{131}I which is organified is transferred to the output of integrator A_4 . Output of this unit represents total organified thyroidal ^{131}I as a function of time. Outputs available from the computer are organified ^{131}I in thyroid, unorganified ^{131}I , and total thyroidal ^{131}I .

Using several plasma decay curves for ^{131}I and various values for unidirectional clearance, K_{TP} and K_{TB} , curves of unorganified and organified thyroidal ^{131}I were recorded for each group of settings.

PREPARATION OF SODIUM PERCHLORATE

The sodium perchlorate was dissolved in sterile distilled water and filtered through a sintered glass pipeline filter. After placing in ampoules, it was autoclaved at 115° for 40 minutes. The solution was quality controlled by passing through an ion-exchange resin (Amberlite IR 120H), eluted with de-ionized water, and the eluate titrated with 0.1 M-NaOH.

APPENDIX 3

MATHEMATICAL DERIVATIONS

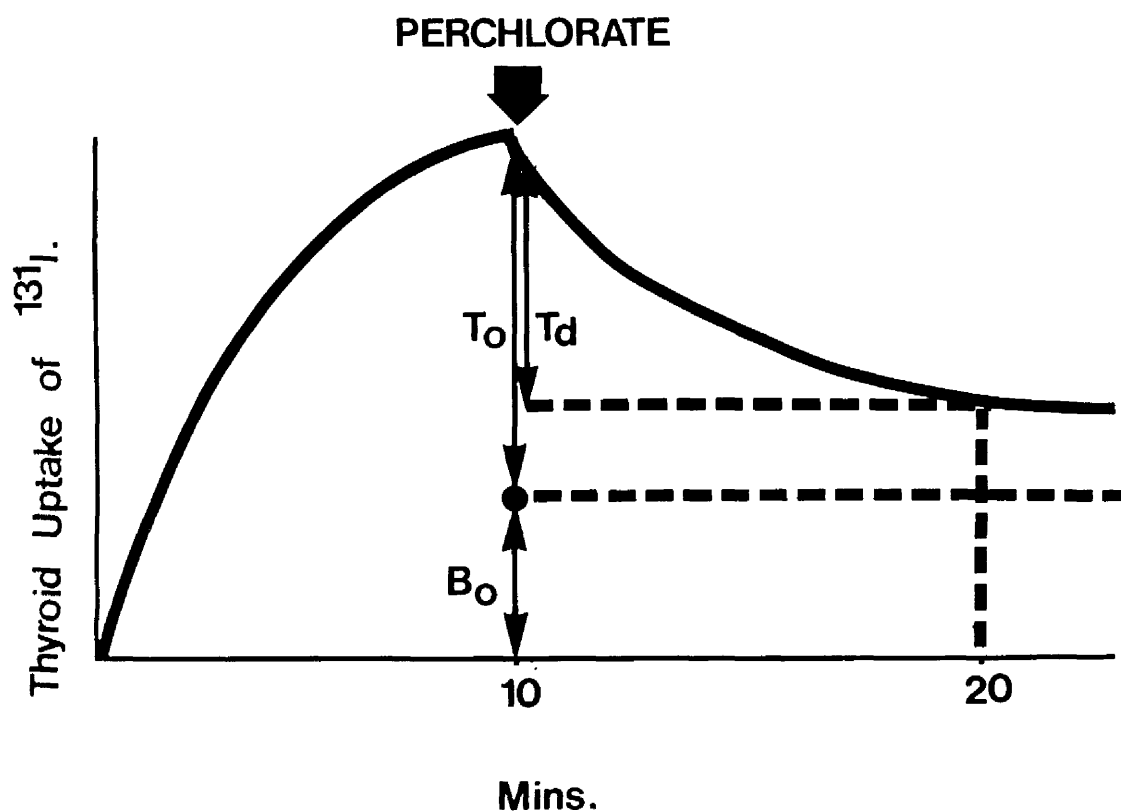


Figure 52: Perchlorate discharge simulation. T_o is the inorganic thyroidal ^{131}I at 10 minutes after IV injection of radio-nuclide and B_o , the organic thyroidal ^{131}I at this time. T_d is the inorganic thyroidal ^{131}I which is discharged from the gland after IV perchlorate administration at 10 minutes.

APPENDIX 3

MATHEMATICAL DERIVATIONS

Perchlorate Discharge Simulation

As shown diagrammatically in Figure 52,

Let T_0 = unorganified thyroidal ^{131}I at 10 minutes

B_0 = organified thyroidal ^{131}I at 10 minutes

T_d = unorganified thyroidal ^{131}I discharged by perchlorate

K'_{TP} = perchlorate induced thyroidal exit-rate constant for ^{131}I

K_{TB} = thyroidal organification rate constant for ^{131}I

From the basic three-compartment model (Chapter 4) and making no assumptions about iodide organification during the perchlorate discharge which commences at 10 minutes,

$$dT/dt = -T (K'_{TP} + K_{TB})$$

$$T(t) = T_0 \cdot e^{-(K'_{TP} + K_{TB})t}, \text{ where } t = \text{time after perchlorate.}$$

$$\text{Now } dB/dt = K_{TB} \cdot T$$

$$B(t) = B_0 + K_{TB} \int_0^t T(t) dt$$

$$B(t) = B_0 + K_{TB} \int_0^t T_0 \cdot e^{-(K'_{TP} + K_{TB})t} dt$$

$$B(t) = B_0 - \frac{K_{TB} \cdot T_0}{K'_{TP} + K_{TB}} \cdot \left[e^{-(K'_{TP} + K_{TB})t} - 1 \right]$$

$$B(t) = B_0 - \frac{K_{TB}}{K'_{TP} + K_{TB}} \cdot T_0 \cdot \left[e^{-(K'_{TP} + K_{TB})t} - 1 \right]$$

This expression $B(t)$ indicates the amount of organified thyroidal

^{131}I at time 't' after perchlorate.

Now sum the organified $B(t)$ and unorganified $T(t)$ thyroidal ^{131}I pools.

$$\begin{aligned} B(t) + T(t) &= T_o \cdot e^{-(K'_{TP} + K_{TB})t} + B_o - \frac{K_{TB}}{K'_{TP} + K_{TB}} \cdot T_o \cdot \left[e^{-(K'_{TP} + K_{TB})t} - 1 \right] \\ &= T_o \cdot e^{-(K'_{TP} + K_{TB})t} - \frac{K_{TB}}{K'_{TP} + K_{TB}} \cdot T_o \cdot e^{-(K'_{TP} + K_{TB})t} + \frac{K_{TB} \cdot T_o}{K'_{TP} + K_{TB}} + B_o \\ &= T_o \cdot e^{-(K'_{TP} + K_{TB})t} \left[1 - \frac{K_{TB}}{K'_{TP} + K_{TB}} \right] + \frac{K_{TB} \cdot T_o}{K'_{TP} + K_{TB}} + B_o \end{aligned}$$

At infinity, when $t = \infty$, $e^{\dots\dots t} = 0$.

Therefore, total thyroidal ^{131}I at $\infty = B_o + \frac{K_{TB} \cdot T_o}{K'_{TP} + K_{TB}}$

Consequently, $T_d(\infty) = T_o - \frac{K_{TB} \cdot T_o}{K'_{TP} + K_{TB}}$

$$T_d(\infty) = T_o \left(1 - \frac{K_{TB}}{K'_{TP} + K_{TB}} \right)$$

$$\frac{T_d(\infty)}{T_o} = 1 - \frac{K_{TB}}{K'_{TP} + K_{TB}}$$

Therefore, if $K_{TB} \xrightarrow{\sim} 0$ after perchlorate administration, $\frac{T_d(\infty)}{T_o}$ will equal 1 since $T_d(\infty) = T_o$.

Now, to obtain $\Delta T_d(t)$, the amount of unorganified ^{131}I still to discharge at 't', we subtract the total thyroidal ^{131}I at ∞ from the total thyroidal ^{131}I uptake at 't'.

$$\Delta T_d(t) = T_o \cdot e^{-(K'_{TP} + K_{TB})t} \left[1 - \frac{K_{TB}}{K'_{TP} + K_{TB}} \right] + \frac{K_{TB} \cdot T_o}{K'_{TP} + K_{TB}} + B_o - \left[B_o + \frac{K_{TB} \cdot T_o}{K'_{TP} + K_{TB}} \right]$$

$$\therefore \Delta T_d(t) = T_o \cdot e^{-(K'_{TP} + K_{TB})t} \left[1 - \frac{K_{TB}}{K'_{TP} + K_{TB}} \right]$$

Divide by $T_d(\infty)$.

$$\frac{\Delta T_d(t)}{T_d(\infty)} = \frac{T_o \cdot e^{-(K'_{TP} + K_{TB})t}}{T_d(\infty)} \left[1 - \frac{K_{TB}}{K'_{TP} + K_{TB}} \right]$$

$$\frac{\Delta T_d(t)}{T_d(\infty)} = \frac{T_o \cdot e^{-(K'_{TP} + K_{TB})t}}{T_o \cdot \left(1 - \frac{K_{TB}}{K'_{TP} + K_{TB}} \right)} \cdot \left(1 - \frac{K_{TB}}{K'_{TP} + K_{TB}} \right)$$

$$\frac{\Delta T_d(t)}{T_d(\infty)} = e^{-(K'_{TP} + K_{TB})t}$$

Therefore, the fractional rate of decrease of T during discharge equals the sum of K'_{TP} and K_{TB} , and

$$\underline{K'_{TP} \text{ (observed)} = K'_{TP} \text{ (actual)} + K_{TB}}$$

I am grateful to Dr. R. Bessent for his assistance in the solution of this problem mathematically.

Calculation of Effective Half-Life of ^{125}I Therapy in Thyroid

The mean biological half-life ($T_{1/2}$) of ^{125}I in 12 patients = 16 days.

Physical half-life of ^{125}I = 60 days.

$$\text{Since } \frac{1}{T_{1/2} \text{ (effective)}} = \frac{1}{T_{1/2} \text{ (biological)}} + \frac{1}{T_{1/2} \text{ (physical)}}$$

$$\text{Mean Effective } T_{1/2} = 12.6 \text{ days.}$$

Calculation of Radiation Dose Deposition in Thyroid

Let $A(t)$ = fraction of therapy radioactivity in gland at time 't'
after 24 hours.

$A(24)$ = fraction of therapy radioactivity in gland at 24 hours.

$D(t)$ = total radiation dose deposited in gland at time 't'.

$T_{1/2}$ = mean effective half-life of ^{125}I in gland.

Then $dD/dt = A(t)$ at time 't'.

$$\text{Since } A(t) \propto A(24) \cdot e^{-\frac{0.693t}{T_{1/2}}}$$

$$dD/dt \propto A(24) \cdot e^{-\frac{0.693t}{T_{1/2}}}$$

$$D(t) \propto A(24) \int_0^t e^{-\frac{0.693t}{T_{1/2}}} dt$$

$$D(t) \propto \frac{T_{1/2}}{0.693} \cdot A(24) \cdot \left[1 - e^{-\frac{0.693t}{T_{1/2}}} \right]$$

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